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Drug Allergy

Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships

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Brian A. Baldo
Formerly-Molecular Immunology Unit
Kolling Institute of Medical Research
Royal North Shore Hospital of Sydney
Sydney, Australia

Nghia H. Pham
Formerly-Molecular Immunology Unit
Kolling Institute of Medical Research
Royal North Shore Hospital of Sydney
Sydney, Australia

ISBN 978-1-4614-7260-5 ISBN 978-1-4614-7261-2 (eBook)
DOI 10.1007/978-1-4614-7261-2
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013937589

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Dedicated to my mother, Sylvia, and the memory of my father, Disma, for their care, guidance, and enduring support and whose hard work, self-reliance, and plain commonsense instructed by example.

BAB

To my father and memory of my mother with love and gratitude. To my wife, Phong-Thuy, and my daughter, Cecile, for their unconditional love, endless support, and encouragement.

NHP

Preface

With its roots in immunology and pharmacology, advancement in the science of drug allergy and its application to clinical medicine has ultimately always been heavily reliant on application of a broad range of investigative methodologies in humans rather than laboratory animal models. The variety of chemically diverse pharmacological agents administered to patients is large and continues to expand and with every new drug released, there is always potential for adverse reactions, some of them allergic. This diversity in chemical structure and pharmacological action together with the range of observed adverse clinical responses; the need to access sufficient numbers of adequately phenotyped patients to study; the necessity of collaborative inputs from laboratory and clinic; and the variety of chemical, cellular, and clinical methodologies needed ensured that progress in the field has generally fallen short of the hoped-for insights. In fact, post the penicillin era when drug allergy was given a much-needed structural perspective, at both the research level and in terms of patient benefits this specialized section of allergic diseases could not be said to have made great advances. There are a number of reasons for this. In the first place, the seemingly perpetual confusion over what constitutes an allergic reaction is something that affects many clinicians as well as the public and the mass media. The term "allergy" continues to be used inappropriately to refer to all sorts of nonimmune-based reactions to a drug and, for many in the medical profession, this reflects a state of mind that is not conducive to reporting/recording, diagnosing, and seeking to understand the true nature of many drug-induced reactions. To reinforce this point, attention is drawn to the 1970s and 1980s when the value of skin testing—prick, intradermal, and patch—although widely advocated and promoted by a few practitioners and aficionados, was not widely understood, appreciated, and applied. Examples were the neglect of the skin test in the diagnosis of drug allergies to anesthetic agents, drugs used in surgery, antibiotics, and other antimicrobial drugs where attitudes to the test sometimes ranged from the uninformed to cynicism of its scientific and clinical relevance. One only needs to talk to anesthetists from that period who were aware of anaphylaxis to neuromuscular blocking drugs to learn of the skepticism of skin testing by many of their professional colleagues. To help overcome this problem, research findings and leadership and instruction, for example, in the form of issued practice parameters and standard operating procedures by the relevant professional bodies, were needed. In anesthesia, this is, in fact, now being

undertaken by Societe Francaises d'Anesthesie et de Reanimation, the Association of Anaesthetists of Great Britain and Ireland, the British Society of Allergy and Clinical Immunology, and the Scandinavian Society of Anaesthesiology and Intensive Care Medicine, all of whom have issued clinical practice guidelines. Over the whole broad area of drug allergy, the European Network for Drug Allergy under the aegis of the European Academy of Allergology and Clinical Immunology has published numerous position papers on allergy practice over the last decade with emphasis, for example, on standardization of methods for the diagnosis of drug allergies.

While acknowledging the sometimes under-appreciation of the problem of drug allergy and the inadequacy of its diagnosis, a third inhibitory factor to progress was probably inevitable. This relates to research directed at identifying underlying mechanisms and improving patient outcomes for cell-mediated drug-induced hypersensitivities such as the various cutaneous reactions ranging from mild exanthemas to severe bullous eruptions. Knowledge of the intricate cellular immune processes involved in antigen recognition, lymphocyte receptor repertoires, and the adaptive immune response as well as recognition of the value and application of a pharmacogenetic approach needed to progress to somewhere near their present levels of understanding before significant inroads could begin to occur. In particular, understanding the role of MHC restriction, drug-specific T cells, and the availability of improved genotyping technologies should significantly increase the chances of advancing knowledge of the cellular hypersensitivity mechanisms and developing new diagnostic and predictive tests. As advantage is increasingly being taken of the results obtained from the extraordinary investigative activity directed at defining cellular and molecular mechanisms of immune processes, chemical approaches, used so effectively in the studies on penicillin and neuromuscular blocking drugs, are being less often utilized as biological and clinical emphases dominate research efforts. The results of this neglect can be seen in the dearth of detail available on the structures recognized by the cellular immune system in delayed hypersensitivity responses. With increasing employment of mass spectrometric characterizations, carefully selected synthetic drug conjugates, and the realization that drugs may be recognized or participate in immune processes in their free state, we can expect that this situation may soon be remedied as investigators seek to expand their current cellular preoccupation, much of it often speculative in nature, with a deeper understanding of the fine structural features that determine allergenic recognition in cell-mediated drug reactions.

With this background and perspective in mind, we set out here to identify the most important culprit drugs implicated in immediate and delayed drug hypersensitivities and to collate up-to-date information on classifications, clinical features, diagnoses, underlying mechanisms, and structure–activity relationships. Chapters dealing with the molecular and cellular mechanisms of drug hypersensitivities, nonimmune-mediated sensitivities, and diagnostic methods are presented as introductory material for in-depth treatises on the β-lactam antibiotics, other antibiotics and antimicrobials, drugs used in anesthesia and surgery, opioid analgesics, corticosteroids, monoclonal antibodies and other biologics, drugs used in chemotherapy, proton pump inhibitors,

iodinated and gadolinium-based contrast media, and nonsteroidal anti-inflammatory drugs. For the latter two groups of drugs where only some of the adverse reactions are truly allergic in nature, discussions have been extended to cover the more dominant and more often seen drug-induced sensitivities or intolerances.

Readers with a historical perspective may be able to detect in this book the influence of two past investigators who made important contributions to hypersensitivity research. Each had widely different professional training, research backgrounds, and clinical involvement, but both were well known for their infectious, unrelenting enthusiasm and the pleasure they derived from pursuing, over many years, original ideas and observations that were very much their own. Time spent by the author in the 1970s in both laboratories left a career-long imprint. In so many ways, the difficult Elvin Kabat in the Columbia University College of Physicians and Surgeons, New York Presbyterian Hospital, and the urbane Jack Pepys at the Brompton Hospital, London, could not have been more different but both were undoubtedly exceptional investigators, one in the laboratory relentlessly applying his quantitative approaches and the other in the world of patients, exploiting the diagnostic potential of, and promoting, one of the simplest technical procedures ever employed in clinical work. The quantitative immunochemical methodologies introduced and developed by the Landsteiner-Heidelberger school of immunochemistry and so expertly applied and propagated by Kabat in his classic text *Kabat and Mayer's Experimental Immunochemistry* (*C. C. Thomas*, *Springfield*, *Il*) influenced a generation of immunologists and maintained a direct line back to Landsteiner and the origins of immunochemistry. By the early 1950s in studies backed by the Office of the Surgeon General, U.S. Army, Kabat had demonstrated a relationship between dextran structures and molecular weight and the propensity of the polysaccharides to provoke systemic allergic reactions. This work ultimately led to a dramatic 90-fold reduction in dextran-induced anaphylactic reactions by pre-dosing with a dextran monovalent hapten. Application of this competitive hapten inhibition strategy, straight out of the Landsteiner–Heidelberger–Kabat quantitative immunochemical protocols, made dextrans easily the safest of all the plasma volume expanders in use. Likewise, Pepys' championing and application of the specificity, sensitivity, and wide applicability of skin prick and provocation testing, despite their apparent simplicity, aided understanding of some important fungal-induced hypersensitivity diseases of the chest, increased appreciation of the clinical value of the procedures, and emphasized their utility for research, diagnosis, and studies of mechanisms in clinical immunology and allergology. Together with his original contributions over many years in the field of occupational allergic diseases studying hypersensitivity pneumonitis (extrinsic allergic alveolitis), his early contributions to our understanding of the late reaction and the training of a constant stream of visiting clinicians from all over the world, Pepys was also fascinated by what often appeared to be hypersensitive responses to "small" molecules including drugs and in his later years he began studies in this area. This was after his earlier pioneering investigations into the sensitizing and allergenic properties of platinum in refinery workers. This work, including the detection

of IgE antibodies to platinum salts, was to prove a forerunner of later interest in patient reactions to the important and heavily used platinum chemotherapeutic drugs. The legacies of Elvin Kabat and Jack Pepys remain apparent today in the originality of their scientific research and value of their clinical contributions. To that can be added the many practitioners in laboratories and clinics who pass on what they themselves learned from the enthusiastic tutelage of these too-often forgotten important early contributors to our knowledge of hypersensitivity states.

In pursuing the authors' own interests and research in drug allergies, some of it recounted in this volume, we would like to acknowledge our enduring collaboration with Dr Malcolm M. Fisher who introduced one of us to the then mechanistically poorly understood problem of perioperative anaphylaxis to what, at the time, were called muscle relaxants. The long-standing clinical interest by Dr Fisher provided all the necessary clinical background and patient material for successful investigations of underlying mechanisms, led on to the study of a range of other drug allergies, and ultimately the development of a useful battery of routine in vitro drug allergy tests. In what was a remarkably small manpower input over many years, we are indebted to Gail Knowland in particular for her long-standing, versatile, and always reliable input into all of the projects, to Dr David Harle for his sustained careful investigations and technical expertise, and, in later years, to Dr. Zhenjun Zhao who, like all his fellow investigators, assiduously pursued the laboratory's quantitative approaches to mechanistic and diagnostic studies on a wide range of poorly understood adverse drug reactions. Dr. John Redmond, Dr. Mary Smal, Dr. Sue Cooney, and Dr. Alistair McCaskill had key roles in the laboratory's research on PAF mentioned here and the development of a sensitive, high-throughput immunoassay for the mediator.

The inclusion in this book of some important photographs and figures was greatly assisted by the generosity and cooperation of Professor S. R. Durham, Dr. D. G. Ebo, Dr. J. S. Fok, Dr. D. Gin, Dr. F. Hasdenteufel, Professor R. J. Heddle, Dr. A. Mar, Dr. P. A. J. Russo, Dr. R. Spiewak, Dr. F. C. K. Thien, and Dr. S. Van Nunen.

Our intention has been to provide a scientifically based textbook with the relevant chemical, immunological, pharmacological, biochemical, and, where appropriate, pharmacogenomic information without losing the clinical perspective that is, in any case, the stimulus and the need for studying drug allergies in the first place. In addition to clinicians, other healthcare professionals, and researchers, the book has been aimed at undergraduate and graduate courses in the biomedical sciences and to serve as a text for students of medicine, pharmacy, nursing, and dentistry.

Finally, as with any subject still beset by many questions, alternative interpretations and different priorities, some analyses, arguments, or conclusions expressed here may not find universal acceptance. In such cases, we remain open and ready to consider all comments in an ongoing effort to improve the book and correct any errors.

Sydney, Australia

Brian A. Baldo
Nghia H. Pham

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Adverse Reactions to Drugs and Drug Allergy: Scope of This Book

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Abstract

In what is essentially a pharmacologically based classification of adverse drug reactions (ADRs), unpredictable and dose-independent drug reactions, designated type B reactions, include hypersensitivity responses while those reactions designated as type A are predictable, dose-dependent, and make up about 80 % of all ADRs. Previous exposure is not always a prerequisite for allergic sensitization, and there are many instances where reactions occur after initial contact with poorly reactive drugs that do not bind to proteins. Risk factors for drug allergy can be divided into those that are patient-related (age, sex, current diseases, previous exposure, genetic factors) and those that are drug-related (nature and cross-reactivity of drug, degree of exposure, route of administration). Genomic studies are already helping to explain some ADRs, for example, the association in Han Chinese of carbamazepine-induced Stevens–Johnson syndrome with HLA-B*15:02 and the association of abacavir hypersensitivity in abacavir hypersensitivity syndrome with HLA-B*57:01. It seems likely that multiple rather than single genes are involved in ADRs. Drug allergy studies promise to provide significant insights into important areas of biomedical investigation including cell recognition and interaction processes, relationships between receptors and effector pathways and mechanisms of mediator actions.

1.1 Adverse Drug Reactions

1.1.1 Definition

A major and seemingly ever-present risk of pharmacotherapy is adverse drug reactions (ADRs). Drug reactions occur frequently and may need expert management. Reactions can be severe and even life-threatening, necessitating the substitution or discontinuation of preferred medications. An additional unwanted clinical response in a sick patient already under treatment constitutes extra burdens for the patient and the managing physician. With approximately 5 % of patients developing adverse reactions during drug therapy and up to 10 % said to react in hospitals, this adds up to a significant public

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_1, © Springer Science+Business Media, LLC 2013

health problem. The often quoted WHO definition of an ADR, published 40 years ago, is "a response to a drug that is noxious and unintended and occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for modification of physiological function." Questioning this definition and in particular the inclusiveness of the wording for minor reactions, Laurence and Carpenter in *A dictionary of pharmacology and allied topics: Elsevier, 1998*, suggested: "A harmful or significantly unpleasant effect caused by a drug at doses intended for therapeutic effect (or prophylaxis or diagnosis) which warrants reduction of dose or withdrawal of the drug and/or foretells hazard from future administration." I.R. Edwards of the Uppsala Monitoring Centre, a WHO collaborating centre for international drug monitoring, and J.K. Aronson of the Radcliffe Infirmary, Oxford, regard the WHO definition as vague, especially with regard to the term "noxious," ask just how minor can an adverse reaction be, query the narrowness of the term "drug" and disagree with what they regarded as ambiguities in other published definitions. To cover these perceived deficiencies, Edwards and Aronson proposed their own succinct definition: "An appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product."

The United States Food and Drug Administration (FDA) states that any serious adverse event should be reported to the FDA and defines such an event as "any undesirable experience associated with the use of a medical product in a patient." The event is said to be serious when the patient outcome is death, life-threatening, hospitalization (initial or prolonged), disability or permanent damage, congenital anomaly/birth defect, required intervention to prevent permanent impairment or damage (devices), and other serious important medical events (e.g., allergic bronchospasm, serious blood dyscrasias, or seizures or convulsions that do not result in hospitalization).

1.1.2 Terminology: Classification of Adverse Drug Reactions and the terms Hypersensitivity, and Allergy

The study of ADRs falls within the discipline of pharmacovigilance. In an early pharmacological classification, ADRs were distinguished primarily on the basis of dose-related and non-dose-related reactions. These two types of reactions were sometimes called types A and B, respectively. Approximately 80 % or more of ADRs are predictable, can be anticipated from the drug's pharmacological actions, are dose-dependent, and resolve when the dose is reduced or withdrawn. Unpredictable reactions, sometimes called idiosyncratic drug reactions, are generally unrelated to the drug's pharmacological actions, are independent of dose, and, even though they usually resolve when treatment is stopped, reactions sometimes progress. From about the early 1980s, three further reaction categories were recognized, one related to dose and time and one classified as delayed but divided into time-related and withdrawal reactions. More recently, a sixth category, "unexpected failure of therapy" has been added. In some classifications of ADRs, a seventh category G, "genetic reactions," is included. This essentially pharmacologically based overall classification of ADRs, together with some distinguishing features and examples of drug reactions, are shown in Table 1.1.

For drug allergy, and for our purposes, an immunological classification is more informative and useful. The unpredictable and dose-independent drug reactions, that is, type B reactions, include the reactions that are said to be hypersensitivity responses but also three categories of nonimmune drug sensitivities termed pseudoallergy, idiosyncratic reactions and type B intolerances. In Fig. 1.1, the four different categories of allergic reactions are referred to as hypersensitivity reactions while the nonimmune (or nonallergic) type B adverse drug reactions are referred to simply as sensitivities. The term "hypersensitivity" is somewhat problematic since it has a long and well-established usage in immunology and allergy but also a history of

Table 1.1 Classification of adverse drug reactions

Reaction type	Examples of reaction	Main features of reaction
A. Augmented pharmacologic effects[a]	– Toxic (intolerant) reactions—e.g., serotonin syndrome to opioids, antidepressants; digoxin toxicity – Side effects—e.g., bronchospasm to β-blocker in hypertensive patient; dry mouth to antidepressants	– Majority of ADRs – Common – Predictable – Usually dose dependent – Related to pharmacologic reaction of drug – Low mortality
B. Bizarre[b] (see Fig. 1.1)	– Immunologic reactions – Idiosyncratic reactions – Pseudoallergy – Intolerance	– Relatively uncommon – Unpredictable – Rarely dose dependent[c] – Unrelated to drug's pharmacologic action – High mortality
C. Chronic (continuous) effects	– Corticosteroid-induced suppression of hypothalmic–pituitary–adrenal axis – Renal papillary necrosis caused by phenacetin	– Uncommon – Cumulative dose and long-term exposure required
D. Delayed effects	– Carcinogenesis – Teratogenesis—e.g., vaginal adenocarcinoma induced by diethylstilbestrol	– Time-related. Apparent some time after drug exposure – Uncommon – Usually dose-related
E. End-of-treatment effects (withdrawal effects)	– Opiate withdrawal syndrome – β-Blocker withdrawal	– Occurs with little or no delay after withdrawal of drug – Uncommon
F. Failure of therapy	– Resistance to drug action—e.g., resistant bacteria to antibiotic or tumor to chemotherapy – Oral contraceptive dose too low	– Common – Usually dose-related – May be caused by drug interactions
G. Genetic reactions[b,d]	– Abnormal drug metabolism due to inherited factors (alleles of P450 (CYP), *N*-acetyltransferase, pseudocholin-esterase) – HLA–drug hypersensitivity associations (e.g., abacavir, carbamazepine, allopurinol) – Succinylcholine sensitivity – Porphyria	– Abnormal drug metabolism appears to be uncommon – Pharmacogenomic studies still in early stages – Ethnicity seems to matter for some drugs, e.g., carbamazepine

[a]Said to account for ~80 % of ADRs
[b]There is evidence that some type B reactions are under genetic control
[c]Exceptions exist, e.g., with type IV hypersensitivity skin reactions; responses to vaccines; desensitization with increasing dosages of drug
[d]ADRs likely to be multigenetic phenomena

meaning different things to different people, and this confusion is still apparent today. In 2001, the European Academy of Allergology and Clinical Immunology (EAACI) published a EAACI Position Statement entitled, in part, "A revised nomenclature for allergy." After setting up a Nomenclature Review Committee to review the EAACI position statement, the World Allergy Organization (WAO) set about promoting globally what was described as acceptable nomenclature for allergic diseases with the ultimate goal of improving communication in the field of allergy. Acknowledging that the nomenclature proposed by the EAACI was based on reaction mechanisms causing the signs and symptoms of allergic disease and these mechanisms were usually inflammatory, the WAO Nomenclature Review Committee issued a revised nomenclature for

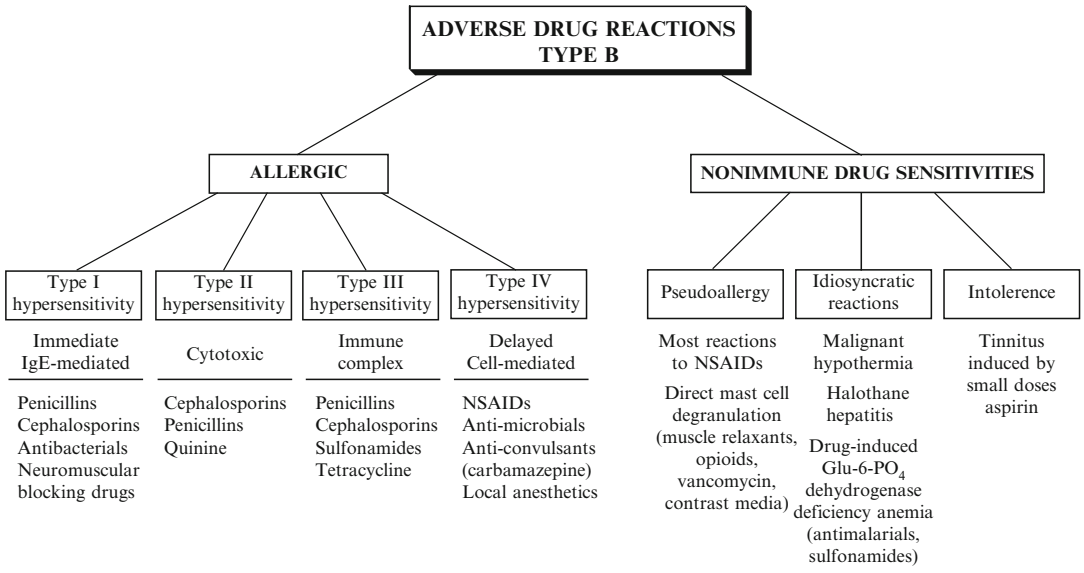

Fig. 1.1 Classification of immune (allergic) and nonimmune sensitivities to drugs. The former are referred to here as hypersensitivities and the latter as nonimmune or nonallergic sensitivities or intolerances. Some drugs commonly involved in allergic reactions and a few examples of nonimmune sensitivities are shown

allergy in 2003. Of its proposed definitions and explanations, those dealing with the terms, hypersensitivity, nonallergic hypersensitivity, and anaphylaxis can be considered contentious. Anaphylaxis, and the proposed definition of it, is discussed in Chap. 2. "Hypersensitivity" was defined as "objectively reproducible symptoms and signs initiated by exposure to a defined stimulus at a dose tolerated by normal persons." "Sensitivity" was said to be an acceptable alternative in special circumstances. This definition of hypersensitivity is inadequate. Apart from the absence of any reference to the adverse nature of a reaction, by omitting any mention of an immunologic mechanism, the description flies in the face of well entrenched, widely accepted, and long-term usage. Firmly established, if not ingrained, use of the labels "immediate hypersensitivity" for IgE antibody-mediated, type I allergic reactions and "delayed hypersensitivity" for delayed-type, type IV, or cell-mediated reactions illustrates how the term hypersensitivity has become synonymous with an immune reaction. This is reinforced by the still accepted and commonly used classification where two other immune mechanisms, antibody-dependent cytotoxic type II and immune complex-mediated type III hypersensitivities, are included with the type I and type IV hypersensitivities in the Gell and Coombs classification of hypersensitivity reactions (see Chap. 2). However, while this classification has served allergists, clinical immunologists, and researchers well for half a century, it is clear that for some adverse drug reactions there are responses that simply do not fit into the four Gell and Coombs categories. Some reactions to contrast media and nonsteroidal anti-inflammatory drugs (NSAIDs) are two examples that readily spring to mind as well as skin reactions such as alopecia, folliculitis, and hyperpigmentation. With some of these drugs, reactions occur that have an immune basis (viz., type I or type IV), that is, they are true hypersensitivities, but in other responses to drugs no immune mechanism can be identified. For NSAIDs, the mechanism underlying most adverse patient responses appears to be drug-induced redirection of mediator synthesis in the arachidonic acid cascade from the cyclooxygenase to the lipoxygenase pathway with no antibody or immune cell involvement (see Chap. 9). Showing some features of a hypersensitivity response and usually presented to an

allergist, clinical immunologist, or dermatologist, it is not difficult to see why such responses are commonly viewed as hypersensitive reactions, but should the long-standing definition of hypersensitivity be changed to accommodate diverse drugs that exert their effects by a number of different mechanisms and where no common humoral or cellular immune basis of action exists?

In defining "allergy," the WAO describe it as "a hypersensitivity reaction initiated by specific immunologic mechanisms." "Drug allergy" should therefore only be used for an ADR where an immunologic mechanism has been established. It was further stated that: "When other mechanisms can be proven, as in hypersensitivity to aspirin, the term *nonallergic hypersensitivity* should be used." If a contrast medium, a NSAID, or any other agent is known not to act via an immune mechanism or if an immune basis of the reaction cannot be established, the NSAID aspirin can indeed help in providing the appropriate terminology, not by the suggested use of "nonallergic hypersensitivity" but by the already commonly employed and clear terms "aspirin sensitivity," "aspirin-intolerant," or "aspirin-induced" (as in asthma). Thus, a patient with asthma induced by (say) celecoxib would simply be described as celecoxib-sensitive, or intolerant to celecoxib, and the condition referred to as celecoxib-induced asthma, celecoxib-intolerant asthma, or celecoxib-exacerbated asthma. A number of late, polymorphic reactions to drugs with mechanisms still to be fully worked out occur several days after administration. Reactions may take the form of maculopapular eruptions, urticaria, fixed drug eruptions, acute generalized exanthematic pustulosis, drug reaction (rash) with eosinophilia and systemic symptoms (DRESS), Stevens–Johnson syndrome, toxic epidermal necrolysis, and vasculitis. Temporal relationships, together with accumulating evidence for activated CD4+ and CD8+ activated lymphocytes from lesions and the generation of drug-specific T cell clones, suggest that these reactions are allergic rather than due to direct toxicity.

In summary, and simply put, there seems to be no compelling reason to alter an established and widely understood definition that is fundamental to the accepted scheme of classification of hypersensitivity states. At present, the common feature for grouping many different reactions with a wide range of signs and symptoms into a broad but unifying classification scheme is the immune basis of each of the responses. Employment of the terminology used for these responses should not be stretched to accommodate reactions that proceed by an entirely different mechanism. It seems likely, however, that many currently inadequately researched and poorly understood drug reactions will probably be shown to be allergic. In the meantime, for reactions to drugs such as NSAIDs, contrast media and many adverse skin responses where it is already clear that immune mechanisms are not involved, or where evidence one way or the other is not yet available, the terms "sensitivity" or "intolerance" should be used instead of "hypersensitivity."

1.1.3 Usage of the Term "Allergy"

Derived from the Greek words allos, meaning other and ergon, meaning work, task, purpose (or ergein, to work), the term allergy was introduced in 1906 by the Austrian pediatrician C.P. von Pirquet who seems to have thought of it as a state of changed reactivity by the host, covering both an increase (hypersensitivity) and a decrease (hyposensitivity) in the allergic response. However, the word was not used to mean hypersensitivity or immunity but as a term for the response that could produce protective immunity on the one hand or hypersensitivity with its detrimental effects on the other. This is different to today's use where the word "allergy" is restricted to specific hypersensitivity to foreign antigens, some of which are also toxic in their own right (such as venoms) and some not (such as foods). It is often said that the medical profession, as well as the public and the media, uses the term allergy inappropriately, loosely, or too casually, and nothing short of a concentrated educational campaign (which is unlikely to happen) will help to overcome this misuse. There is a widespread tendency to consider a large variety of drug-induced adverse systemic and local effects

as allergic in nature due to lazy use of terminology or lack of medical understanding of what constitutes a true allergic response. It is not uncommon, for example, to hear a wide range of different responses to an administered drug, from minor afflictions such as headache, dry mouth, nausea, or syncope to cardiovascular and CNS reactions, described as an "allergic reaction." This needs to be taken into account in any analysis or consideration of drug allergy where a wide coverage of many different drugs of different pharmacological actions may need to be reviewed. In addition to this pharmacological diversity and nonallergic adverse responses, the spectrum of true allergic reactions elicited by drugs can range from a clear type I immediate hypersensitivity reactions manifesting as catastrophic anaphylaxis with all or some of urticaria, angioedema, bronchospasm, and cardiovascular collapse to mild IgE antibody-mediated rhinitis or a mild and transient T cell-mediated rash.

1.2 Drug Allergy

1.2.1 The Early Years

For the still incomplete construct of "Drug Allergy," activity can be seen to have been initiated in 1907 when Wolff-Eisner surveyed the site with the prediction that nonantigenic substances can induce dermatological sensitization after combination with patients' "self" proteins. In the 1920s, Bloch and Steiner-Wourlisch, Wedroff, Mayer, and then Landsteiner and Jacobs (1935, 1936) subsequently cleared the ground and put down the footings by demonstrating sensitization of guinea pigs and humans with simple chemicals. Although the explanation for the formation of antigens from simple reactive chemicals such as acyl chlorides and acid anhydrides seemed satisfying, Landsteiner was aware that for less reactive compounds, for example, quinine, "a chemical interpretation is not immediately to hand." Two explanations were advanced—conversion of unreactive chemicals in vivo to reactive compounds able to combine covalently with protein and what Landsteiner described as a "loose attachment" to protein seen with, for example, salts of heavy metals. Despite the contemporary demonstration of passive sensitization of normal animals to simple chemicals with serum antibodies, the existence of drug-reactive antibodies in subjects allergic to drugs was generally discounted. The prevailing view seemed to be that there was no clear division between drug allergy and other immunological manifestations. It took almost a quarter of a century before the chemist and physician, Bernard Levine and others with their work on penicillins (see Chap. 5), laid solid foundations substantial enough to ultimately support the necessary scientific chemical framework that was to come. Progress was initially slow but now the "finished" edifice promises to be far more complex than Landsteiner and his contemporaries could have imagined as specialist workers with specialized tools of the modern era move in to expand structures and add the necessary, and sometimes surprising, detail.

1.2.2 Drugs, Haptens, and Prior Exposure

From the time of immunology's earliest practitioners, how the immune system recognizes and deals with "small" molecules with molecular weights less than 1 kDa has intrigued researchers. Following earlier suggestions and results indicating that some small molecular weight chemicals can sensitize skin after combination with host proteins, Landsteiner and Jacobs demonstrated a clear relationship between skin sensitizing capacity and the chemical reactivity of haptens to covalently bind to a carrier protein. While Landsteiner's landmark studies on hapten recognition influenced generations of subsequent investigators, recent progress in elucidating mechanisms, both immune and pharmacological, of some different drug intolerances promises to expand our understanding of the body's responses to small antigens free of a macromolecular carrier. Note also the doubtful relevance of delayed (contact) hypersensitivity studies in laboratory

animals to human allergies, especially immediate reactions. In addition, rodents are far from being an ideal animal model for the human allergic state since, apart from the guinea pig, they are not always easy to sensitize and make allergic, the spectra of mediators released and the end organ responses to these mediators often differ from the human responses, and homocytotropic antibodies may differ and are not always of the IgE class. The conundrum of previous exposure is also still with us, and there are numerous examples of allergic reactions to poorly reactive drugs where no protein binding, either by the parent molecule or any putative metabolite or degradation product, can be demonstrated. Landsteiner believed that previous exposure to an antigen is a prerequisite for sensitization and an allergic response but, even acknowledging the possibility of cross-reactions, it is clear that this dogma of prior contact does not always hold. Examples of this keep cropping up as subsequent pages in this book reveal. There seems to be little information on what could be termed innate allergic sensitivity, for example, "natural" IgE antibodies to some allergens (including drugs) but its involvement in some cases seems likely. For drug allergy, the question of whether the allergic sensitivity to a "small" organic molecule (usually prepared by total synthesis and therefore not "natural" in any sense) is genetically determined or derived from natural exposure, remains of great intellectual and practical medical interest. There are already signs, however, that with application of modern genetic technical approaches and insights, including identification of HLA markers for drug-induced hypersensitivities, progress in this area is well underway in an atmosphere of significant, but seemingly justified, expectation.

Just as conjugation of a small nonantigenic molecule such as a drug to a macromolecular carrier is said to be a requirement for allergic sensitization and subsequent reaction, multivalency of the small molecule or hapten is said to be necessary for detecting skin test sensitivity to drugs. In practice, this is not always the case—drugs such as trimethoprim, sulfamethoxazole, opioids (diluted beyond their histamine-liberating concentrations), some contrast media, thiopentone and particularly neuromuscular blocking drugs, and sometimes β-lactams produce positive skin reactions in allergic patients. In fact, the former drugs are routinely used as free drugs in routine diagnosis and some penicillins and cephalosporins sometimes elicit clearer and more specific skin reactions than the corresponding drugs in conjugated form. This subject is considered in forthcoming chapters.

1.2.3 Drug Allergies, Hypersensitivities, and Sensitivities (Intolerances)

In accordance with the definition of allergies possessing an immune basis, an allergic reaction to a drug is mediated by antibody or cells of the immune system. Thus, immediate type I IgE antibody-mediated and delayed-type, type IV T cell-mediated reactions to a drug are considered true drug allergies. Type II antibody-mediated (generally IgG or IgM) cytotoxic reactions with complement involvement to drugs such as penicillins, cephalosporins, quinine, and pyrazolones and type III reactions mediated by soluble immune complexes with antibodies mostly of the IgG class to drugs such as penicillins, sulfonamides, and quinolones are seen as true drug hypersensitivities. Where reactions to drugs proceed with no identifiable immune mediation, such as occurs with nonsteroidal anti-inflammable drugs and contrast media and with direct histamine releasing agents such as opioids and neuromuscular blockers, responses are viewed here as sensitivities or intolerances, not as hypersensitivities and, therefore, not true allergic responses.

With true drug allergies defined as reactions involving immune mechanisms, and bearing in mind the ADRs covering the wide range of reaction types set out in Table 1.1, it is to be expected that the two lists of drugs (immune- verse nonimmune-based) will be significantly different. From a recent survey of nearly 3,700 patient episodes analyzed for ADRs in hospital inpatients in the UK, the drugs most frequently implicated in provoking the reactions were, in order: loop diuretics, opioids, systemic corticosteroids, inhaled

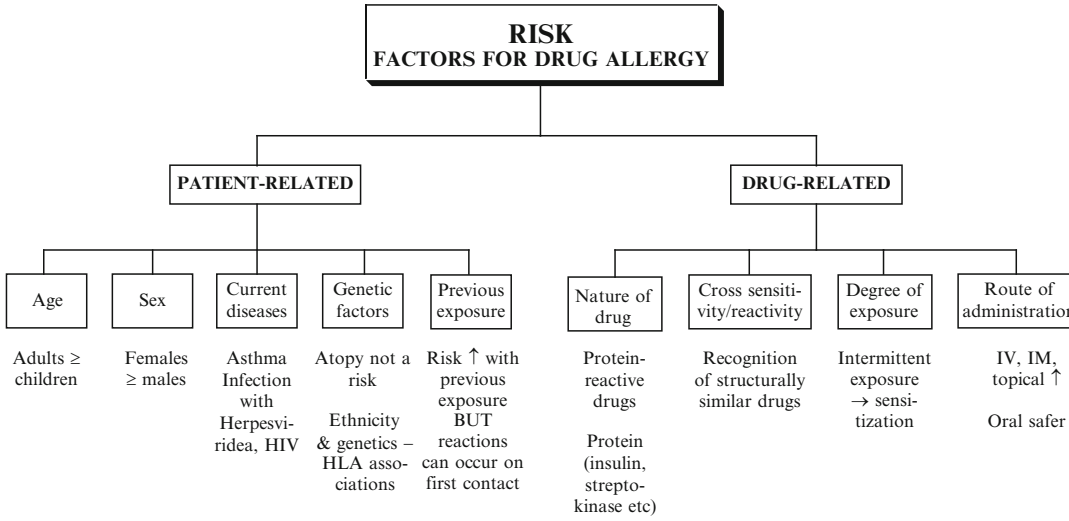

Fig. 1.2 Patient-related and drug-related risk factors for drug allergies

beta-agonists, penicillins, oral anticoagulants, cephalosporins, compound analgesics with opioid, macrolide antibiotics, and low molecular weight heparins. From the above list, a top ten compilation of drugs causing true allergic reactions would include only the penicillins, cephalosporins, possibly heparins, and the compound analgesics with opioid *if* the analgesics were nonsteroidal anti-inflammatory drugs, and perhaps, but not likely, macrolide antibiotics and opioids.

1.2.4 Risk Factors for Drug Allergy

Risk factors for most patients and for most drugs are not always identified or, at best, only vaguely defined, and there is usually little certainty in trying to anticipate a drug reaction. In recent years, significant progress has been made with a very small number of drugs (see, for example, abacavir, Sects. 1.3 and 3.4.2), but these remain the exception. In most cases, some broad risk factors related to the patient and the drug (Fig. 1.2) are considered, but this highlights our ignorance of the subject and represents an early stage in the understanding of the subject of drugs and patient risk. Hypersensitivity reactions appear to be less frequent in children than adults and, in some surveys, less frequent in the elderly. Epidemiological data seem to indicate that female gender is a risk for adverse drug reactions, especially cutaneous reactions, but other surveys dispute this. Asthma is associated with increased risk but the claim that hypersensitivity is more common in systemic lupus erythematosus has not been confirmed. Some drugs, particularly β-lactams like ampicillin and amoxicillin and particularly in children, show a temporal association between drug exposure and rashes. It seems likely, however, that in most cases the reaction is produced by the infectious agent (e.g., in infectious mononucleosis) or by interaction between the drug and the infectious agent. Such responses do not appear to be immunologically mediated. An increased risk of reactions is seen in patients infected with herpesviridae and HIV viruses, good examples being reactions to cotrimoxazole, abacavir, or nevirapine in patients with HIV infection. Atopic patients do not show a higher rate of sensitization to drugs but, once a reaction occurs, they are at increased risk of it being serious, and this is also true for uncontrolled asthmatics and patients with food allergies. A few studies indicate that genetics and ethnicity can be important in certain drug allergies but the situation is certain to be complex with multiple genes as well as environmental factors involved. The search for associations between drug allergies and human leukocyte antigens

(HLA) of the major histocompatibility complex (MHC) on chromosome 6 remains an active area of investigation, and one might expect further discoveries that help to distinguish at risk patients before administration of a potential harmful drug (see example of abacavir below). As so often seen in immune responses, previous exposure to drugs can also induce increased sensitivity and here the question of cross-reactivity needs to be kept in mind (see below). Note, however, as already pointed out, there are many examples of reactions occurring on first exposure as witnessed by reactions to cotrimoxazole, quinolones, muscle relaxants, contrast media, and so on.

When examining drug-related risks, the nature of the drug itself is the first and most obvious consideration. A drug's chemical properties, in particular its molecular weight, structural complexity, and chemical reactivity are relevant. Protein reactivity of a drug is a special consideration, and drugs that are themselves proteins such as insulin, vaccines, biologics such as monoclonal antibodies, interferons, etanercept, aflibercept, anakinra, streptokinase and the anticancer agent *L*-asparaginase are already potential immunogens and allergens. The dose of drug and the duration and frequency of treatment with the drug can affect the risk of reaction. High dosage and prolonged administration may lead to higher risk but again, this is not a hard and fast rule. If anything, intermittent dosage seems more likely to lead to sensitization and hence increased risk. The route of administration of drug can have a marked effect exemplified by the higher incidence of anaphylaxis to a particular drug when given intravenously. Intramuscular administration also carries a higher risk than subcutaneous injection with the oral route being the safest. However, sensitization and severe reactions can also follow oral dosage. Topical application is associated with a high incidence of sensitization and should be completely avoided with some drugs such as the antibiotics chloramphenicol, penicillins, and neomycin. The same is true for sulfonamides. Lastly, cross-reactivity generally has a structural basis and it is unwise to prescribe drugs or change medications without at least a basic knowledge of the chemical structures of the agents. This is particularly important with, for example, the neuromuscular blocking drugs, the β-lactams, sulfonamides, and pyrazolones where clearly similar or identical structural groupings have been implicated as allergenic determinants in allergic recognition. Cross-sensitization may also be important not only from an immunochemical basis but also from the pharmacological action of drugs, for example, NSAIDs where structurally different drugs such as aspirin and oxicams provoke sensitivity to COX-1 inhibitors even though their chemical structures show marked differences.

1.3 The Promise of Pharmacogenomics for understanding and managing Adverse Drug Reactions Including Drug Allergies

The application of genomics, the study of the entire genome of an organism, together with molecular genetics, promises to reduce some adverse drug reactions in the future, improve therapeutic outcomes, and help tailor individual therapies. The variable clinical response to a drug by different individuals is a major problem in medicine since it can lead to both clinical failure and adverse effects in individuals and subpopulations of patients. The study of pharmacogenomics is concerned with the relationship between patients' genes and their responses to drugs. Pharmacogenomics offers one avenue toward personalized medicine aimed at prescribing the optimum drug at the optimum dose for each patient. Pharmacogenetics had its initial impact on drug therapy when the prolonged muscle relaxation of succinylcholine experienced by some patients was explained by an inherited deficiency of a serum cholinesterase, and antimalarial-induced hemolysis was shown to be due to inherited variants of glucose-6-phosphate dehydrogenase (Fig. 1.1). More recently, the discovery of genetic polymorphisms of the drug-metabolizing enzyme cytochrome P450 2D6 or CYP2D6 has led to the discovery of numerous variant alleles at the CYP2D6 locus, many of

which are associated with marked differences in enzyme function. Almost 25 % of drugs are metabolized by CYP2D6 and differences in CYP2D6 activity and drug clearance of up to 40-fold can lead to severe adverse effects and patients who do not respond to their drug therapy.

Some examples of how pharmacogenomic studies can provide invaluable insights into ADRs are provided by the discoveries of the strong association in Han Chinese of carbamazepine-induced Stevens–Johnson syndrome and the HLA-B*15:02 allele and myelotoxicity seen in thiopurine methyltransferase-deficient patients on azothioprine. Such discoveries have led to the US FDA recommending genetic testing of patients prescribed certain drugs and the employment of labels containing pharmacogenomic information. Abacavir hypersensitivity syndrome (AHS) provides another fascinating example of the contribution of genetic factors to the predisposition of some individuals to drug-induced hypersensitivity reactions. Abacavir, a reverse transcriptase inhibitor administered to patients infected with human immunodeficiency virus-1 (HIV-1), can provoke a potentially fatal multiorgan response, AHS, involving fever, skin rash, and gastrointestinal and respiratory symptoms in about 2–8 % of patients. Investigations revealed a strong association between abacavir hypersensitivity and the well-defined 57.1 MHC ancestral haplotype which comprises HLA-B*57:01, HLA-DR7, and HLA-DQ3 with an odds ratio greater than 100. Although early results indicated that HLA-B*57:01 held promise as a screening test to prevent AHS, some cases of AHS proved negative for the HLA allele and low sensitivities of it for AHS were seen in nonwhites. However, screening studies suggested that HLA-B*57:01 screening can largely eliminate AHS, and patch testing was found to distinguish patients with true immunologically mediated AHS from false positives. It is now known that HLA-B*57:01 has a negative predictive value of 100 % for AHS confirmed by patch testing across both white and black populations, and HIV treatment guidelines recommend HLA-B*57:01 screening as part of the routine care of patients before being prescribed abacavir. An explanation of why abacavir is tolerated by 45 % of patients positive for HLA-B*57:01 is still to be found but, in the meantime, a genetic screening test has been implemented globally as part of primary HIV clinical practice. Basic studies in the laboratory have revealed that AHS is specifically restricted by HLA-B*57:01 and mediated by CD8+ lymphocytes. This classic example of what has become a successful translation of pharmacogenomics into the clinic under the direction of lead investigator Simon Mallal in Western Australia serves as a prototype study for other drugs where genetic testing might be utilized for the prevention of drug reactions. The recently elucidated molecular basis of AHS is covered in Chap. 3, Sect. 3.4.3.

In an interesting evaluation of the potential role of pharmacogenomics in reducing the incidence of ADRs, 22 variant allele articles were reviewed and matched with 27 drugs frequently implicated in ADRs from 18 ADR studies. Fifty nine percent of the drugs are known to be metabolized by at least one enzyme with a variant allele that causes poor metabolism whereas only 7–22 % of drugs randomly selected are metabolized by a variant allele. The authors concluded that drug therapy suited to an individual's genetic makeup might lead to significant reductions in ADRs. As a logical extension of such findings, a proposal to collect and utilize pharmacogenomic information after regulatory approval of a drug has been put forward. As part of surveillance after a drug's launch, patients taking the drug would lodge a biological specimen (e.g., a blood spot) and undergo a genetic scan. DNA comparisons between patients with and without ADRs would then identify genetic markers that could be used to identify patients at risk of an ADR to the drug. The pharmacogenomic approach to ADRs is now extending to some of the most important medications with, for example, new information on the frequently prescribed statin drugs and warfarin, the anticoagulant widely used to prevent thrombosis and thromboembolism. The SLCO1B1 gene on chromosome 12 encodes a transporting polypeptide that regulates hepatic uptake of statins. Recently, common variants in SLCO1B1 that are strongly associated with an

increased risk of statin-myopathy have been identified and an algorithm for estimating the appropriate warfarin dose on both clinical and genetic data has been developed.

Note, however, that it now seems more likely that multiple rather than single genes are involved in ADRs, and this may add greatly to the difficulties of ultimately successfully applying these approaches to the everyday clinical situation.

1.4 Improvements In Drug Delivery and the Allergenicity of Drugs

With increasing usage of different carrier systems to improve drug solubility, delivery, or stability, selected drug modifications may give rise to some unanticipated and unwanted consequences as well as the hoped-for improvement(s). As more drug carriers such as cyclodextrins, dendrimers, vesicles like liposomes and niosomes, liquid crystals, soluble polymers, and micelles formed by self-assembly of amphiphilic block copolymers, cell ghosts, and hydrogels are employed for a wider variety of drugs, carrier-induced changes including chemical modifications, exposure, masking of structural groupings, and altered orientation of groups may occur. This will carry with it the possibility of antigenic and allergenic changes as well as the possibility of an additional allergenic contribution by the carrier molecule. Widely and heavily used drugs such as β-lactams, neuromuscular blockers, and many other commonly used oral, injected, and topical medicaments are already being formulated and/or marketed as drug-carrier complexes. With the cyclodextrins alone, there were more than 45 drug-cyclodextrin inclusion complexes approved in 2010. Allergenic modification of the neuromuscular blocker rocuronium by a chemically modified γ-cyclodextrin used to sequester the drug from the neuromuscular junction to reverse neuromuscular blockade is discussed in Sect. 7.4.6. The possibility of change in the allergic recognition of a known allergenic drug as well as appearance of allergenic activity in drugs previously not known to provoke allergic reactions needs to be kept in mind in the planning of preclinical drug safety assessments of those drugs modified by efforts to improve drug delivery.

1.5 Scope of This Book

1.5.1 The Place of Drug Allergy in Immunology

Drug allergy has never been a topic given much coverage by textbooks of immunology and the subject, if considered at all by general allergy texts, is often restricted to penicillin with no, or scant, details provided on other drug allergies. In two of the currently most widely studied and respected immunology textbooks used at undergraduate and graduate level and by medical students, coverage in one is restricted to three sentences on anaphylaxis to penicillin and, in the other, to a short paragraph on the involvement of penicillin in IgE-mediated reactions and type II hypersensitivity responses. In the latter case, the name of the only other drug mentioned is found in the sentence: “Another drug that is known to provoke anaphylaxis is the anesthetic hexamethonium.” To some extent, this situation can be understood since many insights into mechanisms of the allergic responses to a whole range of different drugs have occurred only in recent years. Many of the well-established advances, even simply the names of important culprit allergenic drugs (e.g., cephalosporins, anesthetic agents, and chimeric monoclonal antibodies), let alone details of their allergenic properties, have yet to find their way into the general immunological and even some of the allergological literature. Ultimately, improvements in diagnosis and treatment should result from increased understanding of the mechanisms underlying immediate and delayed reactions and some nonimmune drug intolerances. In the process, fundamental insights into the recognition and handling by the immune system of small (<1 kDa) usually synthesized, nonprotein, non-carbohydrate molecules are likely to be gained and to enter the mainstream immunological literature.

1.5.2 The Need for a Single Text Book on Drug Allergy

At the research level, drug allergy is a mixture of chemistry, immunology, pharmacology, and biochemistry with increasing inputs in the laboratory from immunochemistry, cell biology, molecular biology, and molecular genetics. Although in the early development of research on the immune response to chemicals, animal models were instrumental in the discovery and subsequent study of contact sensitivity and delayed type hypersensitivity, allergy research has never been far removed from human patients and the clinical application of its findings. Reports of advances in the understanding of diagnosis and treatment of allergic reactions to drugs are likely to be found in journals in any of the above-mentioned disciplines as well as journals devoted to allergy and the general medical literature. With significant recent advances in our understanding of the mechanisms underlying the induction, development, and manifestations of the various allergic and other drug sensitivities, now seems to be an appropriate time to review, in one volume, the research and clinical developments made since the first relevant publications appeared just over a century ago. In other words, there is a need for an integrated text that treats the subject in its own right. In doing this, we have pursued a straightforward approach that can be summarized as follows:

- Classification and description of the various drug-induced hypersensitivity and sensitivity responses.
- Presentation of current knowledge of the mechanisms underlying the various systemic and cutaneous drug reactions including mechanisms of immediate, late, and delayed reactions, type II and type III hypersensitivities, and some important drug-induced sensitivities lacking an immune basis.
- A comprehensive review of diagnostic methods.
- In depth discussions of those groups of drugs most frequently implicated in reactions—the β-lactams; other antibacterials including antibiotics, sulfonamides, trimethoprim, and chlorhexidine; anesthetic agents and drugs used in surgery; NSAIDs; opioid analgesics; iodinated and gadolinium-based contrast media; therapeutic monoclonal antibodies and some recombinant biologics used for therapy; corticosteroids; a wide variety of drugs with anti-neoplastic properties used in cancer therapy; and proton pump inhibitors.
- Where appropriate, concentrations of drugs for skin and challenge testing, photographs of examples of important drug-induced cutaneous and cutaneous/systemic reactions, structural information, and relevant metabolic pathways are presented. Treatments of drug-induced reactions are beyond the scope of this book and are not considered.
- At the conclusion of each chapter a "Further reading" list of seminal/authoritative/innovative publications is provided. In some cases, an early seminal work, a particularly informative and/or comprehensive review, or an advanced treatment of a subject is included.

Summary

- With approximately 5 % of patients developing ADRs during drug therapy and up to 10 % reacting to drugs in hospitals, ADRs are a significant health problem.
- In what is essentially a pharmacologically based classification of ADRs, six or seven different categories are recognized. Approximately 80 % of reactions are predictable, dose dependent, and resolve upon withdrawal of drug. Unpredictable reactions are generally unrelated to the drug's pharmacological action, are independent of dose, and usually resolve when treatment stops.
- Unpredicted and dose-independent drug reactions, known as type B reactions, include hypersensitivity responses.
- "Allergy" is a much misused term. The well entrenched and widely accepted term "hypersensitivity" is reserved here for immune-based reactions. Some nonimmune reactions such as adverse reactions to NSAIDs and contrast media are referred to as sensitivities or intolerances.

- The foundations of modern allergy research and practice were laid at the end of the nineteenth and first quarter of the twentieth centuries and extended by the work of Landsteiner and collaborators with their demonstration of the sensitization of laboratory animals and humans with simple reactive chemicals.
- Previous exposure is not always a precondition for allergic sensitization. There are numerous examples of reactions to poorly reactive drugs where no protein binding to the parent drug, metabolites, or degradation products can be demonstrated.
- Type II antibody-mediated cytotoxic reactions and type III reactions mediated by soluble immune complexes are seen as true hypersensitivity reactions.
- Risk factors for drug allergies can be divided into those that are patient-related or drug-related. The former group covers the influences of age, sex, current diseases, previous exposure, and genetic factors; the latter, cross-sensitivity/reactions of drugs, nature of the drug, the degree of exposure, and route of administration.
- The application of genomics promises to better explain and ultimately reduce some ADRs. Results so far have demonstrated the association in Han Chinese of carbamazepine-induced Stevens–Johnson syndrome with the HLA-B*15:02 allele and the association of abacavir hypersensitivity in AHS with HLA-B*57:01.
- As a result of the latter findings, genetic screening is now part of primary HIV clinical practice.
- It seems likely that multiple rather than single genes are involved in ADRs.
- Efforts to improve drug delivery may lead to loss, or enhancement, of a drug's former allergenicity, or the appearance of allergenicity in a drug previously not implicated in allergic reactions. This will need to be recognized in preclinical assessments of drug safety.
- Studies of drug allergies have the potential of providing insights into some important areas of biomedical investigation including cell recognition and interactive processes, relationships between receptors and effector and signaling pathways, mechanisms of action of a variety of soluble mediators, and the genetic bases of many adverse reactions to drug molecules
- Drug allergy has been neglected in text books of immunology and the time is right for the subject to be presented in detail and with wide coverage of its many aspects.

Further Reading

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Classification and Descriptions of Allergic Reactions to Drugs

2

Abstract

Four types of hypersensitivities may be distinguished. Type I, or immediate hypersensitivity, occurs within about 30 min, is IgE antibody-mediated, and the allergic signs and symptoms are triggered by cross-linking of mast cell-bound IgE which leads to mast cell degranulation and release of inflammatory mediators. Drugs well known to cause type I reactions include β-lactams, neuromuscular blockers, and some NSAIDs. Anaphylactoid reactions may mimic the signs and symptoms of anaphylaxis but, unlike the latter reactions, anaphylactoid reactions are not immune-mediated. Clinical manifestations of anaphylaxis include erythema, urticaria, angioedema, bronchospasm, and cardiovascular collapse. Urticaria is often associated with angioedema and anaphylaxis. ACE inhibitors are responsible for one in six hospital admissions for angioedema. Types II and III hypersensitivities are known as antibody-dependent cytotoxic and immune complex-mediated hypersensitivities, respectively. Examples of drug-induced type II reactions are hemolytic anemia, thrombocytopenia, and granulocytopenia. A serum sickness-like reaction is the prototype type III drug hypersensitivity. Type IV drug hypersensitivities are mediated by antigen-specific T cells. Reactions occur 48–72 h after antigen exposure and are therefore referred to as delayed. Examples of delayed cutaneous reactions include allergic contact dermatitis, psoriasis, FDE, AGEP, DRESS, SJS, and TEN.

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_2, © Springer Science+Business Media, LLC 2013

2.1 Hypersensitivity: The Early Years—From Koch to Gell and Coombs

The phenomena that constitute the basis of hypersensitivity reactions were discovered, and studies initiated, in the approximately 20-year period between Robert Koch's demonstration of a delayed hypersensitivity reaction to the tubercle bacillus in 1882 and the demonstrations of anaphylactic, Arthus, and serum sickness reactions in the first decade of the twentieth century. Having discovered the tubercle bacillus, Koch showed that the delayed inflammatory response to an intradermal injection of the organism could indicate previous exposure to *Mycobacterium tuberculosis* in an asymptomatic person. This response became known as the tuberculin reaction and, as the Mantoux test, is recognized today as the classical and best known example of a delayed hypersensitivity reaction. Continuing studies initiated with P. Portier in 1902, Charles Richet by 1907 had shown that numerous proteins in small doses could provoke not "phylaxis" or protection, but "anti-protection" or what he termed "anaphylaxis," and this state could be passively transferred to a normal animal by serum. In the first demonstration of the reaction which came to bear his name, N.M. Arthus in 1903 produced erythematous and edematous reactions in rabbits after repeated injections of horse serum, and in 1906, von Pirquet and Bėla Schick demonstrated the immune complex-mediated serum sickness reaction (see Sects. 2.2.3 and 3.8) so named because it sometimes occurred following the administration of antisera prepared in horses (e.g., anti-pneumococcal antisera). Despite Landsteiner's initial conviction that cell-bound antibody was the cause of contact sensitivity, Chase's experiments in 1941–1942 transferring "sticky," unclarified peritoneal exudate from guinea pigs sensitized by hapten–stromata conjugate with Freund's complete adjuvant led to the discovery that viable cells from the peritoneum, lymph nodes, and spleen-mediated contact sensitivity and tuberculin reactivity. It was then a further 21 years before Gell and Coombs produced their classification of hypersensitivity reactions based on the immune mechanisms underlying the different reactions, that is, the latency of reactions, humoral or cellular involvement, complement involvement, and pathophysiological consequences for the host.

2.2 Classification of Hypersensitivity Reactions

A number of arguments have been voiced over the years against the Gell and Coombs classification and published criticisms include its alleged too narrow focus on the deleterious consequences to the host, that some antigens such as drugs do not fit well into the categories, and that the classification scheme should be simplified to pseudo-allergic, antibody-mediated, and cell-mediated reactions. More recently, subdivisions have been suggested for type IV reactions. We believe that the existing classification has deficiencies but, with mechanism-based categories, it remains, overall, the simplest, most valid, and most logical way of distinguishing the host's immune sensitivities. Four types of hypersensitivity reactions, designated types I, II, III, and IV, are distinguished.

2.2.1 Type I Hypersensitivity

Type I hypersensitivity is also known as immediate, or sometimes anaphylactic, hypersensitivity. Responses usually occur within 30–60 min but can be extremely quick (within minutes) and dramatic as in anaphylaxis. In some cases a late onset reaction may occur about 3 or 4 h after allergen exposure and the immediate reaction. This late phase reaction generally peaks at about 6–12 h and subsides at about 24 h (see Sect. 3.3). Immediate reactions can affect a single organ such as the skin (urticaria, eczema), eyes (conjunctivitis), nasopharynx (allergic rhinitis), mucosa of mouth/throat/tongue (angioedema), bronchopulmonary tissue (asthma), and gastrointestinal tract (gastroenteritis) or multiple organs (anaphylaxis), causing symptoms ranging from minor itching and inflammation to death.

Immediate hypersensitivity is mediated by IgE antibodies interacting with mast cells and basophils (Sect. 3.2) and with eosinophils, platelets, and neutrophils amplifying the response. IgE antibodies bind to their complementary FcεRI receptors on mast cells via their Cε3 region leaving both antibody combining sites free to interact with the complementary allergenic determinants. This interaction causes cross-linking of the cell-bound antibodies leading to degranulation of the anchoring mast cells and release of the variety of preformed, and then newly formed, mediators of inflammation and hypersensitivity.

Drugs well known for causing type I allergic reactions include penicillins, cephalosporins, quinolones, chlorhexidine, neuromuscular blocking drugs, some nonsteroidal anti-inflammatory drugs (NSAIDs) such as pyrazolones, trimethoprim, sulfamethoxazole, proton pump inhibitors, heparin, insulin, *L*-asparaginase, etanercept, and chimeric human–animal monoclonal antibodies used for therapy, but it is uncommon to find a drug at least moderately used that has not provoked an anaphylactic reaction in at least one rare individual.

2.2.1.1 Anaphylaxis to Drugs

It seems that every new drug administered to humans has the potential to provoke an anaphylactic response, and the likelihood of such a response therefore increases with increased administration. Unfortunately, while the signs and symptoms of what is commonly termed "anaphylactic shock" seem clear enough, incidences of anaphylaxis in different countries and to different drugs, as well as the terminology used for reactions is often far from consistent.

2.2.1.1.1 Terminology

For many years, two terms, "anaphylaxis" and "anaphylactoid," have been used to describe relatively rare reactions that have features commonly associated with severe immediate, often life-threatening, allergic reactions. These two terms are distinguished by the underlying mechanisms of the reactions. The term anaphylaxis is used by many for an immune IgE antibody-mediated, systemic immediate type I hypersensitivity reaction, often occurring within seconds or minutes, involving the release of potent allergic and inflammatory mediators from mast cells and basophils and producing at least some of the signs and symptoms of severe immediate reactions such as cardiovascular symptoms (tachycardia, hypotension, cardiovascular collapse), respiratory involvement (dyspnea, bronchospasm, wheeze), gastrointestinal symptoms (nausea, vomiting, abdominal pain), and cutaneous manifestations (urticaria, angioedema, erythema, pruritus). With the knowledge that some agents induce anaphylaxis via an IgG antibody-mediated mechanism, for example, as occurs in some responses to dextrans (and an IgG-mediated pathway to anaphylaxis is known in some laboratory animals such as mice), the wording "IgE antibody-mediated" in the definition above is more correctly replaced by, simply, "antibody-mediated." The term anaphylactoid is used for reactions that may show clinically similar or even identical signs and symptoms but where no immune-mediated mechanism can be shown, for example, reactions caused by direct mast cell degranulation induced by drugs such as vancomycin, opioids, or contrast media.

In 1998 in the USA, a Joint Task Force on Practice Parameters for Allergy and Immunology defined anaphylaxis as an "immediate systemic reaction caused by rapid, IgE-mediated immune release of potent mediators from tissue mast cells and peripheral basophils." Anaphylactoid reactions were seen as mechanistically different and said to "mimic signs and symptoms of anaphylaxis, but are caused by non-IgE-mediated release of potent mediators from mast cells and basophils." By 2004 when the National Institute of Allergy and Infectious Diseases (NIAID) and the Food Allergy and Anaphylaxis Network (FAAN) cosponsored a symposium on the definition and management of anaphylaxis, the existing mechanistic definition of anaphylaxis was judged to be "of marginal utility" to the physician and other health care personnel when faced with "the variable constellation of signs and symptoms of this disorder." In a second symposium in 2005 the NIAID and FAAN brought together allergists, immunologists, pediatricians, emergency and intensive care physicians, internists, and pathologists

together with representatives from the professional bodies in Canada, Europe, and Australia to (among other aims) work toward a universally accepted definition of anaphylaxis and establish clinical criteria to accurately identify cases of anaphylaxis. The definition of anaphylaxis that emerged from this gathering was: "Anaphylaxis is a severe, potentially fatal, systemic allergic reaction that occurs suddenly after contact with an allergy-causing substance." For a definition that would be useful to both the medical and lay communities, anaphylaxis was defined as "a serious allergic reaction that is rapid in onset and may cause death." For the researcher, and those practicing clinical medicine but perhaps not for the layman, it is hard to see how these definitions are an improvement or more useful than the former ones.

As mentioned in Chap. 1, in 2003, the Nomenclature Review Committee of the World Allergy Organization (WAO) defined anaphylaxis (which they referred to as an "umbrella term") as "a severe, life-threatening generalized or systemic hypersensitivity reaction." It was further stated that "the term *allergic anaphylaxis* should be used when the reaction is mediated by an immunological mechanism, e.g., IgE, IgG, and immune complex complement related" and, "an anaphylactic reaction mediated by IgE antibodies, such as peanut-induced food anaphylaxis, may be referred to as *IgE-mediated allergic anaphylaxis*." Lastly, "anaphylaxis from whatever nonimmunologic cause should be referred to as *nonallergic anaphylaxis*." As with the WAO suggested changes to the definition of hypersensitivity and introduction of the term "non-allergic hypersensitivity," (see Chap. 1, Sect. 1.1.2), the proposed definition of anaphylaxis is not only unnecessary but inadequate and the distinction of anaphylaxis into "IgE-mediated allergic" and "nonallergic" is needlessly complicating, cumbersome, and redundant. To move away from a mechanistic definition in defining anaphylaxis for professionally trained workers in medicine and science in an age when scientific progress is influencing clinical medicine to an unprecedented extent, seems to be a step backward. There seems to be much to gain by encouraging physicians to think more mechanistically of their art. If anaphylaxis is defined in terms of being a systemic, rapidly proceeding, immune-based immediate reaction involving potent mediators released from mast cells and basophils and producing a range of clearly defined profound clinical effects, the addition of descriptors such as "allergic," "IgE-mediated allergic," and "nonallergic" to the word anaphylaxis becomes completely unnecessary. Anaphylaxis has, over many years, come to be known as an allergic reaction mediated by IgE. Hence, the terms "allergic anaphylaxis," "IgE-mediated allergic anaphylaxis" and "nonallergic anaphylaxis" each have added words that are redundant or are a contradiction in terms. It then follows that "anaphylactoid" is a suitable and convenient term to cover those immediate systemic reactions that mimic anaphylaxis but are not immune-mediated and, with the stipulations and distinctions outlined above in mind, reactions will be referred to either as anaphylactic or anaphylactoid throughout this book.

2.2.1.1.2 Incidence of Anaphylaxis

The incidence of all causes of anaphylaxis in Western countries is estimated to be from about 8 to 50 per 100,000 persons per year with 3–4 % hospitalized and a lifetime prevalence of 0.05–2 %. In one retrospective Danish study of anaphylaxis over a 13-year period outside hospital in a catchment area of 48,000 subjects, an incidence of 3.2 cases per 100,000 persons per year was found. Of the 20 cases of anaphylaxis identified, seven were provoked by oral penicillin and three by oral aspirin, indicating that anaphylaxis to penicillin in the non-hospital environment was more common than thought. A retrospective population-based cohort study of 1,255 US residents in one county over a 5-year period revealed an incidence of anaphylaxis of 30 per 100,000 persons per year with an average annual incidence rate of 21 per 100,000 person-years. Drugs, along with foods and insect stings, were the main causes. In a prospective study of 432 Australian patients, medication was the cause in 8.3 % of cases. Minimum occurrence and incidence of new cases were estimated to be 12.6 and 9.9 episodes per 100,000 patient-years,

respectively. An analysis of anaphylaxis admissions to UK critical care units between 2005 and 2009 revealed 1,269 adult and 81 pediatric admissions representing 0.3 % of admissions to adult units and 0.1 % of admissions to pediatric units. The data showed that hospital admission rates for anaphylaxis have increased sevenfold in the last two decades and the absolute numbers of both adults and children rose year-on-year. The authors remarked that drug-triggered reactions are more common in older people and concluded that each UK critical care unit is likely to see at least one anaphylaxis case per year.

A large proportion of anaphylactic reactions is due to drugs. In hospitalized patients the prevalence is said to be 3 in 10,000 with deaths occurring in 3–9 % of patients. The overall incidence and the mortality of anaphylaxis induced by drugs are not known but figures are available for some individual drugs or groups of drugs in some localities. Most data over the years for incidences of anaphylaxis to a single drug (or group of drugs) have been for penicillins with published estimates of approximately one reaction for every 10,000 prescriptions and 15–40 reactions per 100,000 persons. During the 1960s and 1970s penicillins were often claimed to be the most common cause of drug-induced anaphylaxis in the US and that may still be the case today. Reactions to contrast media and blood-volume replacement fluids have been reported in one of every 600 and 400 persons, respectively, receiving the drugs. More detail of incidences of anaphylaxis to other drugs are to be found in the chapters on the different drug groups. In a population-based case–cohort study in The Netherlands, a drug was found to be the causative agent in 107 of 252 cases of suspected anaphylaxis classified as "causal relationship certain" or "causal relationship probable." Of the 107 cases, 19 % were caused by the NSAID glafenine, 11 % by amoxicillin, 7 % for each of diclofenac and acetaminophen and 6 % for propyphenazone. In fact, at least 42.6 % of the 107 cases of possible/probable cases of anaphylaxis were due to a NSAID (27 miscellaneous drugs each causing one reaction were not named). Perhaps the most reliable data available on incidences of drug-induced anaphylaxis are the figures for drugs used perioperatively. Published incidences of anaphylactic and anaphylactoid reactions include 1 in 5,000–13,000 for Australia, 1 in 1,250–5,000 for New Zealand, 1 in 3,500 for England, 1 in 5,000 in Thailand, and 1 in 4,600 in France. By far the biggest contributor to anaphylaxis alone (that is, with anaphylactoid reactions not involved) during anesthesia is the group of neuromuscular blocking drugs. Reported incidences to these drugs are 1 in 10,000–20,000 Australia, 1 in 5,500 France, 1 in 5,200 Norway, 1 in 10,263 Spain, 1 in 5,500 Thailand and approximately 500 cases per year in the UK. Of the drugs implicated in anaphylaxis, the most complete and reliable data on incidences of reactions are those obtained from studies of perioperative anaphylaxis in Australia and France. A breakdown of the main drugs responsible for anaphylaxis during anesthesia in Australia and France shows the following incidences of reactions (as percentages, Australian figures first): neuromuscular blockers 61.9, 58.1; induction agents 10.4, 2.3; antibiotics 8.6, 12.9; colloids 4.6, 3.4; opioids 2.6, 1.7 (see also Table 7.1).

2.2.1.1.3 Clinical Features of Drug-Induced Anaphylactic Reactions

In the Dutch study discussed above in which 43 different drugs were implicated in causing at least one anaphylactic reaction, the main symptoms seen in the patients admitted to hospitals were (in decreasing order of occurrence) erythema, angioedema, hypotension, bronchospasm, pruritus, urticaria, nausea/vomiting, tachycardia, loss of consciousness, diarrhea, upper airways symptoms, conjunctivitis, laryngeal edema, abdominal pain, and bradycardia. Erythema was seen in 57 % of patients, angioedema in 51 %, hypotension in 36 %, bronchospasm and pruritus in 35 %, urticaria in 31 %, tachycardia in 19 %, and bradycardia in 4 %. For drug-induced anaphylactic and anaphylactoid reactions, however, it is the perioperative data accumulated over many years by Fisher and Baldo in Australia and by the Laxenaire and Mertes groups in France (see Fig. 7.2) that is the most informative. As emphasized by the former workers, the list of

Table 2.1 Clinical features in 315 patients with life-threatening anaphylaxis to anesthetic drugs

	Total		Worst feature		Sole feature	
Clinical features	Number	%	Number	%	Number	%
Cardiovascular collapse	283	89.8	251	79.7	33	10.5
Bronchospasm						
Severe	52	16.5	52	16.5	10	3.2
Transient	50	15.9				
Angioedema	73	23.2	4	1.3	4	1.3
Urticaria	43	13.7				
Rash	42	13.3				
Erythema	151	47.9				
Gastrointestinal symptoms	30	9.5				
Pulmonary edema	11	3.5	3	1.0	1	0.3
Generalized edema	15	4.8				

Other major common features: vomiting, diarrhea, laryngeal edema
Uncommon major features: cardiac failure, disseminated intravascular coagulation, hemoptysis, melena
Minor features: rash, flush, rhinitis, cough, lacrimation, urticaria, pruritus, aura, conjunctivitis
Late features: headache, thromboembolism, edema, wound hematoma, vaginal discharge
Data adapted from Fisher M, Baldo BA. Eur J Anaesthesiol 1994;11:263 and Med J Aust 1988;149:43

complete signs and symptoms (Table 2.1) does not appear in every patient; cardiovascular collapse is the most common symptom and usually the worst; in addition to cardiovascular collapse there is usually at least one other sign; asthmatic patients who experience anaphylaxis usually get bronchospasm; and a transient bronchospasm or difficulty in inflating the lungs is often seen as the first sign of a reaction. Note that when the problem of lung inflation persists, it is often the most difficult feature to reverse. Cardiovascular collapse, the most common life-threatening feature, is due to vasodilation and the pooling of peripheral blood which reduces the venous return and the cardiac output. Whether the heart is a target organ in anaphylaxis in humans as it is in some other animals is still not clear. Cardiac failure occurs in anaphylactic patients with cardiac disease but rarely in patients with normal cardiac function. Table 2.1 indicates that when bronchospasm is severe, it is usually the worst feature and the sole feature in about 20 % of the cases. Bronchospasm may be critical since the high pressures needed for inflation reduce venous return and may increase ventricular compliance. Angioedema, which involves the head, neck, and upper airways, usually progresses slowly and it is therefore prudent to observe the patient for at least 12 h. Gastrointestinal symptoms include severe abdominal pain, vomiting, diarrhea, hematemesis, and melena and last up to 6 h. Noncardiogenic pulmonary edema, which occasionally occurs as a single clinical feature of anaphylaxis and is a common postmortem finding in resultant deaths, has been reported as a sole delayed reaction to protamine after cardiac bypass surgery.

2.2.1.1.4 Clinical Grading System for Anaphylaxis

Definitions of anaphylaxis vary, sometimes making it difficult to interpret and compare clinical findings. Classification of anaphylactic reactions on the bases of clinical features observed and their severity is obviously needed, but there has been no uniformity or agreement on the relevance and importance of the parameters to be included in any grading system. Some grading systems are simple descriptions of common and/or important symptoms while others are based on statistical analyses of individual reaction features, sums of allotted scores, the appearance of "two or more" clinical features, or cardiovascular compromise. From case records of over 1,100 acute generalized hypersensitivity reactions, logistic regression analyses of associations between reaction features and hypotension and hypoxia were used to con-

Table 2.2 Grading system for hypersensitivity reactions including anaphylaxis

Grade	Broad clinical features	Defining symptoms and signs
1 MILD	Cutaneous and subcutaneous only	Generalized erythema, periorbital edema, urticaria, or angioedema
2 MODERATE	Cardiovascular, respiratory, or gastrointestinal involvement	Dyspnea, stridor, wheeze, nausea, vomiting, dizziness, diaphoresis, chest or throat tightness, or abdominal pain
3 SEVERE	Hypoxia, hypotension, or neurologic compromise	Cyanosis or $SpO_2 \leq 92$ % at any stage, hypotension (systolic BP < 90 mmHg in adults), confusion, collapse, loss of consciousness, or incontinence

Adapted from Brown SGA. Clinical features and severity grading of anaphylaxis. J Allergy Clin Immunol 2004;114:317 with kind permission from Elsevier Limited

struct a grading system suitable for defining reaction severity in clinical and research settings. Three grades that correlated with epinephrine usage, mild, moderate, and severe (Table 2.2), were discerned from the clinical features of confusion, collapse, unconsciousness, incontinence, diaphoresis, vomiting, presyncope, dyspnea, stridor, wheeze, and nausea, all of which were associated with documented hypotension and hypoxia. The clinical features in the moderate and severe grades fit in with a definition of anaphylaxis. A major difference between this and other grading systems is the identified importance of gastrointestinal symptoms. A possible limitation of this study and its conclusions is that clinical assessments were undertaken by emergency medicine clinicians rather than allergists, so confirmatory skin and IgE antibody tests are lacking.

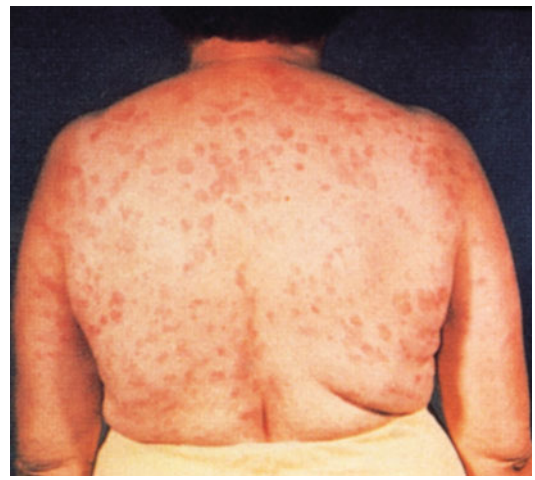

Fig. 2.1 A case of severe generalized chronic urticaria and nonlife-threatening angioedema unresponsive to antihistamines. From Fox R, Lieberman P, Blaiss M. Centralized urticarial and angioedema and angioedema of the face. Atlas of allergic diseases; 2002;IS:08. With kind permission from Springer Science+Business Media B.V

2.2.1.2 Urticaria and Angioedema

Urticaria or hives, the second most common cutaneous reaction induced by drugs after exanthematous reactions, occurs often in association with angioedema, in cases of anaphylaxis and in serum sickness. Virtually any drug can cause urticaria. Hives are generally raised, circumscribed, erythematous papules and plaques (Fig. 2.1) with a central area of pallor, often round in shape and of variable size. Erythematous areas may be smooth surfaced, patchy, or confluent and generalized (Fig. 2.2). Outbreaks that may occur anywhere on the skin are extremely pruritic and transient, usually appearing within 36 h of drug exposure and resolving without sequelae within 24 h. On rechallenge with drug, lesions may appear within minutes. Lesions that persist longer than 24 h and which are painful, burning, or leave bruising and/or pigmentation changes may indicate urticarial vasculitis. Acute urticaria that is more common in children, may appear early after exposure (perhaps minutes) and can last several weeks; chronic urticaria, more common in adults, occurs in episodes that last longer than 6 weeks. Drugs are only infrequently implicated in cases of chronic urticaria and, in fact, no external agent or disease state has been implicated in 80–90 % of patients with chronic urticaria. The incidence of chronic urticaria is said to be as high as 1 % in the USA and several other countries, but the figure is likely to be closer to about 0.1–0.3 %. The mechanisms involved in the

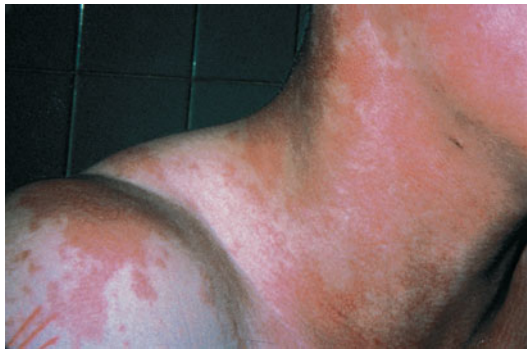

Fig. 2.2 An example of generalized urticaria (hives) showing smooth, erythematous, and pruritic confluent papules and plaques. Aspirin and other nonsteroidal anti-inflammatory drugs are a frequent cause of hives but virtually any drug can precipitate the condition. From Anderson J, Lieberman P, Blaiss M. Allergic drug reactions. Atlas of allergic diseases; 2002;IS;26. With kind permission from Springer Science+Business Media B.V

spectrum of urticarial reactions are reviewed in Sect. 3.2.8. Drugs implicated in reactions include those that provoke type I IgE-mediated reactions (e.g., β-lactams, other antibiotics, antimicrobials such as sulfonamides and trimethoprim, neuromuscular blockers, pyrazolones); direct mast cell degranulation (e.g., opioids, contrast media, vancomycin, quinine, pentamidine, atropine); drugs that promote or exacerbate urticaria due to their pharmacological effects on metabolic pathways (angiotensin-converting enzyme [ACE] inhibitors, e.g., captopril, enalopril, lisinopril; NSAIDs, e.g., aspirin, indomethacin, ibuprofen); drugs involved in type III immune complex formation as in serum sickness (e.g., amoxicillin, cefaclor, ciprofloxacin, monoclonal antibodies); excipients, preservatives, coloring agents, antioxidants (e.g., benzoic acid, sulfites, tartrazine, butylated hydroxytoluene). Note that evidence for adverse effects, including the induction of urticaria, is often lacking for implicated agents in the latter group.

Angioedema (Quincke's edema), which is seen less often than urticaria but often accompanies it, is a vascular reaction resulting in swelling of the face (Fig. 2.3) around the mouth, in the mucosa of the mouth, throat and tongue, eyelids, genitalia, and occasionally involving the hands and elsewhere (Fig. 2.1). Swelling, which can be itchy and painful, is the result of increased permeability and

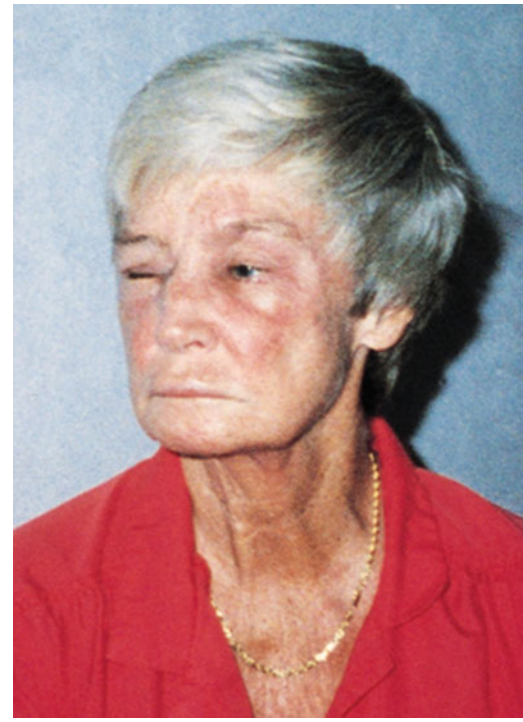

Fig. 2.3 Angioedema of the face showing non-pruritic swelling of the cutaneous tissues with some erythema. Angioedema persists longer than urticaria due to the accumulated fluid in the tissues. From Fox R, Lieberman P, Blaiss M. Centralized urticarial and angioedema and angioedema of the face. Atlas of allergic diseases; 2002;IS:08. With kind permission from Springer Science+Business Media B.V

leakage of fluid which produces edema of the submucosal tissues, deep dermis, and subcutaneous tissues. As a result of compression of nerves, patients may experience decreased sensation in the affected areas. In severe reactions involving the airways, stridor, gasping, and wheezing may indicate the need for tracheal intubation. As with urticaria, angioedema is often an IgE antibody-mediated reaction to a drug and one of its main causes is the ACE inhibitor group of drugs which induce reactions with an incidence of 0.1–0.5 %. For black Americans and Afro-Caribbeans this incidence is three times higher. ACE inhibitors are the responsible agents for approximately one in six patients admitted to hospital for treatment of angioedema. It is thought the drugs increase bradykinin levels in peripheral tissues, and this leads to the rapid fluid accumulation

seen in angioedema (Sect. 3.2.8.5.2). Angioedema to ACE inhibitors usually occurs within the first week of treatment and the drug should be withdrawn immediately in the patients who experience a reaction. Aspirin and other NSAIDs are other common causes of angioedema generally involving the face. Responses to the NSAIDs may be complex with mixed cutaneous and respiratory symptoms (Sect. 9.5.2).

Unlike the other types of hypersensitivity caused by antibodies, namely types II and III, only a proportion of the population, the so-called atopics, have a predisposition to developing a type I hypersensitivity reaction when exposed to the allergen in question. Atopy may have a genetic component but type II and III reactions like, for example, penicillin-induced hemolytic anemia and serum sickness, may occur in all individuals, and this may result without a prior sensitization phase.

2.2.2 Type II Hypersensitivity

Type II hypersensitivity, also known as cytotoxic hypersensitivity and antibody-dependent cytotoxicity, causes reactions that are serious and potentially life-threatening. A number of different organs and tissues may be affected with the involvement of multiple underlying mechanisms. The antigens involved are often endogenous but drugs can attach to cell membranes provoking drug-induced immune hemolytic anemia, thrombocytopenia, and granulocytopenia, all examples of type II reactions. Reaction times can range from minutes to hours; IgM or IgG antibodies mediate the reactions and in antibody-dependent cellular cytotoxicity, target cells coated with antibody are killed by a non-phagocytic process involving Fc receptor-bound leukocytes (NK cells, monocytes, neutrophils, and eosinophils). Drug-induced hemolytic anemias with IgG antibodies and complement-mediated cytotoxicity (or an autoantibody) implicated may occur after treatment with penicillins, quinidine, α-methyldopa, and some cephalosporins. In the penicillin-induced condition, an atypical anti-penicillin antibody (perhaps with drug-surface protein specificity) appears to be involved. Drug-induced thrombocytopenia with quinine, quinidine, propylthiouracil, gold salts, acetaminophen, vancomycin, and sulfonamides in immune complexes adsorbed onto platelet membranes is well documented. Other mechanisms are also operative in immune-mediated thrombocytopenia. Cytotoxic antibodies to pyrazolone drugs, thiouracil, sulfonamides, anticonvulsives, and phenothiazines may produce granulocytopenia by the destruction of peripheral neutrophils. For more detail, including mechanisms, of each of these reactions, the reader is referred to Chap. 3, Sect. 3.7.

2.2.3 Type III Hypersensitivity

Type III hypersensitivity, also called immune complex hypersensitivity, is mediated by soluble immune complexes of antigen with antibodies mostly of the IgG class but sometimes IgM. Deposition of immune complexes in tissues results in a tissue reaction initiated by complement activation that may lead to mast cell degranulation, leukocyte chemotaxis, and inflammation induced by the cell influx. After exposure, reactions may develop over a period of about 3–10 h against antigens that can be endogenous as in DNA/anti-DNA/complement deposits in the kidneys of patients with systemic lupus erythematosus or, more often, exogenous as in the Arthus reaction in rabbits to intradermal injection of soluble antigen or intrapulmonary Arthus-like reactions in humans to inhaled antigen associated with farmer's lung and extrinsic allergic alveolitis. Other exogenous antigens eliciting type III responses include those from organisms such as filarial worms, dengue virus, and microbial antigens abruptly released following chemotherapy in patients with high antibody levels. More importantly for our purposes are type III reactions with drug involvement where examples include erythema nodosum leprosum in the skin of leprosy patients treated with dapsone, the Jarisch–Herxheimer reaction in syphilitic patients treated with penicillins, and serum sickness-like reactions caused by a number of different drugs including penicillins, cephalosporins, sulfonamides, ciprofloxacin,

tetracycline, lincomycin, NSAIDs, carbamazepine, phenytoin, allopurinol, thiouracil, propanolol, griseofulvin, captopril, gold salts, barbiturates, and monoclonal antibodies.

For a description of the immunological events central to the mechanism of serum sickness, see Sect. 3.8. As well as the most common cause of classical serum sickness reactions, namely equine antisera given as an antitoxin, other foreign proteins such as vaccines, anti-lymphocyte globulins, streptokinase, and hymenoptera venoms have also been implicated in reactions. Drugs can cause a reaction that is clinically similar to the protein-induced condition although nonprotein antigens generally do not induce the response. Symptoms typically show up 6–21 days after drug administration and are similar to those seen in the classical reaction. Lymphadenopathy is common and fever, one of the earliest signs of serum sickness, tends to persist throughout the illness. Note, however, that lymphadenopathy has not been reported in penicillin-induced serum sickness. Cutaneous symptoms occur in up to 95 % of patients with urticarial and morbilliform eruptions being the most common and erythema and petechiae sometimes appearing. Angioedema may be seen and arthritis or arthralgia and gastrointestinal symptoms of cramping, nausea, vomiting, or diarrhea occur in up to 67 % of patients. Joints (knee, ankle, shoulder, elbow, wrist, spine, jaw) may be severely affected; respiratory symptoms and splenomegaly occurs; and hepatomegaly, peripheral neuropathies, encephalomyelitis, and pericarditis have been reported. With few if any laboratory-detected changes to help with the diagnosis, serum sickness-like reactions, as with classic serum sickness, tend to be diagnosed clinically. Although the drug-induced reaction can be severe, most are mild and usually resolve spontaneously within a few days or weeks after discontinuing the drug. True incidences of the reaction to various drugs are not known although amoxicillin and amoxicillin–clavulanic acid were involved in 18 % of serum sickness-like reactions examined in a pediatric emergency department, and there are numerous reports implicating cefaclor in children. Serum sickness-like reactions are said to make up 4 % of all adverse drug reactions to amoxicillin. Hypersensitivity vasculitis is another example of a type III hypersensitivity response that may be induced by drugs. Drugs most commonly implicated include β-lactams, cotrimoxazole, NSAIDs and some monoclonal antibodies (see Chap. 11, Fig. 11.1).

2.2.4 Delayed-Type (Type IV) Hypersensitivity

Unlike types I, II, and III hypersensitivities that proceed with the involvement of antibodies, type IV hypersensitivity reactions are cell-mediated, in particular, by antigen-specific effector T cells. The term "delayed" refers to the cellular response that generally becomes apparent 48–72 h after antigen exposure and distinguishes the response from type I or immediate reactions that often appear within minutes and peak in a matter of minutes or just a few hours. Type IV hypersensitivity is not represented by a single reaction. Rather, it is a number of related responses seen in a variety of reactions that may have beneficial or undesirable consequences for the host and which, at first sight, do not seem to have a lot in common except for their cellular immune base. Apart from the prototypic tuberculin test, these different reactions include cellular responses to intracellular pathogens such as mycobacteria, fungi, and parasites; graft rejection; graft verse host reactions; granulomatous inflammation as occurs in Crohn's disease and sarcoidosis; tumor immunity; some autoimmune reactions; and in contact allergy and allergic contact dermatitis. Other infectious diseases in which type IV reactions play a part include leprosy, histoplasmosis, toxoplasmosis, blastomycosis, and leishmaniasis. A well-known example of allergic contact dermatitis is the reaction provoked by the lipid-soluble chemicals, mixed pentadecacatechols in urushiol oil present in plants of the *Rhus* genus, namely, poison ivy, poison oak, and poison sumac. After crossing the cell membrane, the catechol derivatives interact with intracellular proteins before binding with MHC (major histocompatibility

complex) class I molecules. These modified peptides are recognized by CD8+ T cells which respond with the secretion of cytokines such as IFN-γ and cell destruction.

In some classifications, type IV reactions are subdivided into three categories based on the time of onset, the clinical manifestations, and the cells involved. The main features of the different categories in one such classification include:

1. Tuberculin type reaction (seen as local induration): antigen presented intradermally; reaction time 48–72 h; cells involved, lymphocytes, monocytes, and macrophages
2. Contact type reaction (seen as eczema): cutaneous contact (e.g., with chemicals, poison ivy, nickel); reaction time 48–72 h; cells involved, lymphocytes, macrophages
3. Granulomatous type reaction (as in leprosy): antigen persists in the host; reaction time 21–28 days; cells involved, macrophages, epitheloid, and giant cells

Another subdivision of type IV hypersensitivity reactions has categories distinguished by the effector cells and mediators involved together with the resulting associated cutaneous reactions. Four subdivisions are defined: type IVa, mediated by monocytes with IFN-γ as dominant cytokine; type IVb, mediated by eosinophils with IL-5 and IL-4 involvement; type IVc, mediated by T cells with perforin and granzyme B as important effector molecules; and type IVd, mediated by neutrophils with IL-8 involvement. Representative skin reactions for the so-called types IVa, b, c, and d are, respectively, maculopapular rash, maculopapular rash with eosinophilia, Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and acute generalized exanthematous pustulosis (AGEP).

It should be remembered that for delayed, type IV cutaneous allergic drug reactions (and sometimes for type I reactions as well), the allergic response is generally heterogeneous with overlapping reactions and with the involvement of different effector cells including various T cells with separate functions. Naturally, this heterogeneity affects the clinical picture and adds to the difficulties of the clinician in coming to a confident diagnostic conclusion.

Table 2.3 presents a side-by-side summary of all four hypersensitivities according to the Gell and Coombs classification viewed from the perspective of the responses to drugs in humans.

Delayed cutaneous hypersensitivity reactions to drugs generally begin from about 7 to 21 days after contact with the drug. Subsequent reactions may appear only 1 or 2 days after reexposure. Identification and specificity of the drug is established from oral challenge studies, patch tests, and intradermal tests read after a delay of at least 48 h. Different T cell subsets with their individual profiles of cytokines and chemokines are associated with different skin hypersensitivity reactions although there is often overlapping cytokine involvement.

Summarized descriptions of the most important immune-mediated delayed cutaneous adverse drug reactions follow. Readers should refer to Sect. 3.6.3 for details of the mechanisms involved in these reactions.

2.2.4.1 Allergic Contact Dermatitis

Contact hypersensitivity may result from sensitization to chemicals such as chromates and picryl chloride in industrial settings; from chemicals such as *p*-phenylenediamine in hair dyes; nickel in jewelry, glasses, and devices (Fig. 2.4); urishiol in *Rhus* plants; and drugs such as neomycin (Sect. 6.1.5.1; Fig. 6.7) and bacitracin (Sect. 6.1.5.2) used in topical applications. Contact dermatitis is the most common occupational disease although it should be remembered that not all contact dermatitis has an immune basis; some irritants such as detergents, solvents, acids, and alkalis may provoke irritant contact dermatitis. Allergic contact dermatitis may appear at any age. The time between the initial exposure to the offending agent and the development of skin sensitivity may only be 2–3 days while the interval between exposure and the first symptoms may be as early as 12 h but is usually closer to 48 h. The reaction is generally confined to the contact site but generalized reactions may occur. When the face is involved, swelling of the eyelids is common. Other skin sites may become involved by the patient touching other areas of skin after touching the

Table 2.3 Hypersensitivities to drugs according to the Gell and Coombs classification[a]

Type of hypersensitivity	I	II	III	IV
Other designations	Immediate; anaphylactic	Cytotoxic	Immune complex	Delayed; cell-mediated; T cell mediated
Time for reaction to reach maximum	Seconds to 30 min[b]	Hours (~1 day)	3–10 h	24–72 h
Immune reactant(s)	IgE antibody	IgG (and IgM) antibodies	IgG antibody (and/or IgM)	Th1, Th2 and/or Th17 cells Cytotoxic lymphocytes
Effector mechanism	Mast cell and basophil activation	Complement fixation Phagocytes, NK cells (Fc receptor cells)	Complement Phagocytes	Macrophage activation Cytotoxic lymphocytes Eosinophil activation
Intradermal response to antigen	Wheal and flare	Lysis and necrosis	Erythema and edema	Erythema and induration
Histology	Degranulated mast cells; Cellular infiltrates including neutrophils[b]	Immunofluorescence shows antibody, complement, neutrophils	Acute inflammatory reaction; Mainly neutrophils	Perivascular inflammation; Mainly mononuclear cells
Sensitivity transferred by	Serum IgE antibody	Serum antibody	Serum antibody	Lymphoid cells
Examples of disease states	Erythema; urticaria; angioedema; respiratory symptoms; GI symptoms; anaphylaxis	Drug-induced hemolytic anemia, thrombocytopenia, agranulocytosis (immune form)	Serum sickness; Drug-induced vasculitis	Allergic contact dermatitis; Psoriasis; Maculopapular exanthema; AGEP; FDE; DRESS; SJS; TEN; EM
Drugs implicated	β-Lactams; other antibacterials; NMBDs; some NSAIDs; quinolones; mAbs; proton pump inhib's	β-Lactams; quinine; quinidine; sulfonamides; NSAIDs; procainamide; gold; carbamazepine; propylthiouracil; ticlopidine	β-Lactams; ciprofloxacin; sulfonamides; lincomycin; tetracycline; NSAIDs; carbamazepine; allopurinol; gold; methyldopa; mAbs	NSAIDs; β-lactams; other antibiotics; anti-convulsants; antimalarials; local anesthetics; barbiturates; quinolones; dapsone

[a]Coombs RRA, Gell PGH. In: Gell PGH et al. (eds.) Clinical Aspects of Immunology. Oxford: Blackwells. 1975, p. 761–81
[b]Late reaction may occur ~3–4 h after immediate reaction, peak at ~12 h and subside by ~24 h
AGEP acute generalized exanthematous pustulosis, *DRESS* drug reaction with eosinophilia and systemic symptoms, *EM* erythema multiforme, *FDE* fixed drug eruption, *mAbs* monoclonal antibodies, *NMBDs* neuromuscular blocking drugs, *NSAIDs* nonsteroidal anti-inflammatory drugs, *SJS* Stevens–Johnson syndrome, *TEN* toxic epidermal necrolysis

affected area. The skin may be red, swollen, and show blistering or be dry and bumpy but in the active stage, the skin usually shows redness, with raised areas and blisters (Fig. 2.5).

2.2.4.2 Psoriasis

Psoriasis is a chronic, immune-mediated inflammatory skin disease with a prevalence generally stated to be around 2 % but, depending on the population, this figure can range from zero in Samoa to as high as 4.8 % in the USA (US range 0.6–4.8 %). Ethnicity appears to be involved, for example, American blacks show a prevalence of only 0.45–0.7 % and, likewise, there appears to be a genetic predisposition to psoriasis illustrated by a concordance rate of 70 % in monozygotic twins and prevalences of 50 % and 16 % respectively when both parents or only one has psoriasis. Several genetic susceptibility loci have been reported, particularly the so-called psoriasis susceptibility 1 (PSORS1) locus on chromosome 6 which appears to be associated with up to 50 % of cases of psoriasis. As well as drugs, psoriasis may be triggered by smoking, alcohol, and withdrawal of systemic or topical corticosteroids. One study showed 23 % of patients were taking more than three medications and 11 % of these were taking more than ten medications. The clinical

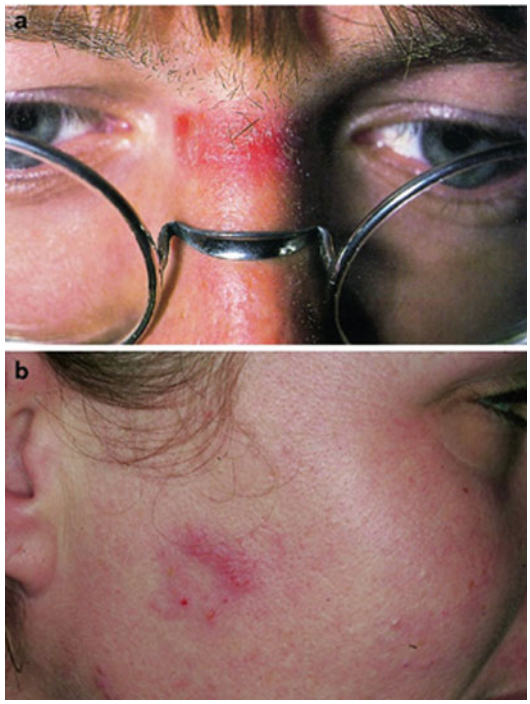

Fig. 2.4 Allergic nickel contact dermatitis caused by (**a**) reading glasses and (**b**) a multifunction key on a cell phone. From Veien NK, in: Johansen JD, Frosch PJ, Lepoittevin J-P, editors. Contact Dermatitis. 5th ed. Berlin: Springer-Verlag; 2011. With kind permission from Springer Science+Business Media

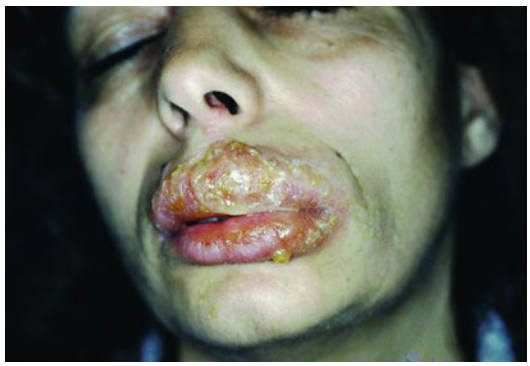

Fig. 2.5 Acute allergic contact dermatitis to the topical antiviral tromantadine hydrochloride showing blistering. From Brandão FM, in: Johansen JD, Frosch PJ, Lepoittevin J-P, editors. Contact Dermatitis. 5th ed. Berlin: Springer-Verlag; 2011. With kind permission from Springer Science+Business Media

presentation of the disease is, typically, sharply demarcated erythematous papules and rounded plaques covered by silvery micaceous scales most commonly on the scalp, elbows, umbilicus, lumbar region, and knees (Fig. 2.6). Lesions of psoriasis vulgaris may show small pustules but various forms of pustular psoriasis including generalized and localized variants have been described. Both the more common vulgar form and the pustular form may progress to psoriatic erythroderma affecting the whole body. Fingernails and toenails can also be affected in all types of psoriasis.

A relationship between psoriasis and certain drugs is well recognized. The authors of one study, for example, suggested that acute generalized exanthematous pustulosis (AGEP) is a cutaneous reaction pattern that might be favored by a "psoriatic background," and medications could be responsible for psoriasis in up to 83 % of cases. Drugs can affect psoriasis in a number of ways—they may induce the disease, cause skin eruptions, induce lesions in previously unaffected skin in patients with psoriasis, and induce a form of psoriasis that is resistant to treatment. Drugs that provoke psoriasis can be divided into two categories—drugs that induce psoriasis but withdrawal of the drug stops further progression of the disease and drugs that aggravate psoriasis but the disease still progresses even after drug withdrawal. Lithium, β-blockers, and synthetic antimalarials are the drugs most commonly mentioned in triggering or worsening psoriasis, but there are many other drugs that have been implicated either as inducers of the disease or for provoking eruptions. In the former case, the list includes acetazolamide, aminoglutethimide, amiodarone, amoxicillin, ampicillin, aspirin, chloroquine, cimetidine, corticosteroids, cyclosporin, diclofenac, diltiazem, hydroxychloroquine, indomethacin, lithium, methicillin, propranolol, and terbinafine. According to Litt (2006), there are at least 125 different drugs known to be responsible for the eruption or induction of psoriasis. Psoriatic eruptions occur in 3.4–45 % of patients treated with lithium. The mechanism is currently believed to be by inhibition of the intracellular release of calcium as a result of lithium-induced

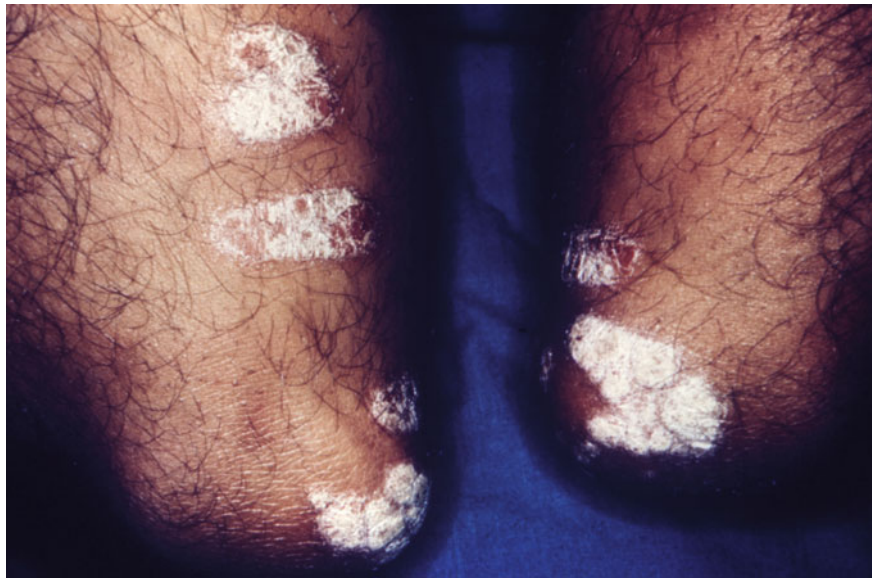

Fig. 2.6 Psoriasis of the elbows showing irregular red patches covered by dry, scaly hyperkeratotic stratum corneum (image by courtesy of the Center for Disease Control and Richard S. Hibbets)

depletion of inositol monophosphatase. Supporting this is the beneficial effect of inositol supplementation in lithium-provoked psoriasis. The administration of the antimalarials chloroquine and hydroxychloroquine to patients with psoriasis is considered to be contraindicated by some. Exacerbation of psoriatic lesions and the induction of the disease have been found after use of these drugs and chloroquine has been implicated in resistance to treatment when given as an antimalarial. Clinical improvement is seen after withdrawal of β-blockers in cases where the drugs have induced or exacerbated psoriasis. The reactions to β-blockers usually manifest 1–18 months after initiation of therapy although, because of some histological findings and clinical features (such as absence of elbow and knee involvement and lesions that are less thick and scaly), some believe that the condition induced by these drugs is not true psoriasis. The mechanism underlying the reactions induced by β-blockers is thought to involve blockade of epidermal β_2 receptors, which leads to decreased cAMP levels in the epidermis and ultimately keratinocyte hyper-proliferation. NSAIDs, many of which are available over the counter, are frequently used by patients with psoriasis or psoriatic arthritis, but it has been claimed that the NSAIDs are the most common cause of both the induction and exacerbation of psoriasis. Naproxen in particular has been implicated. NSAIDs exhibit a short latency period of only about 1.6 weeks. Leukotrienes, which increase due to NSAID-induced redirection of the arachidonic acid prostanoid pathway to the lipoxygenase pathway (see Chap. 9. Sects. 9.4.1 and 9.5.2.1.2), are thought to aggravate psoriasis. Other drugs for which there is enough data to be sure that they are a significant problem for psoriasis include ACE inhibitors, amiodarone, benzodiazepines, cimetidine, clonidine, digoxin, fluoxetine, gemfibrozil, gold, interferons, quinidine, and tumor necrosis factor. ACE inhibitors appear to be a problem in patients over 50 years of age.

Although the treatment and management of drug reactions are beyond the scope of this book, it is worth noting that a number of biological agents are currently being used either in the clinic or experimentally to treat psoriasis. These include etanercept, a fusion protein inhibitor of tumor

necrosis factor (see Sect. 11.2), and the chimeric and human monoclonal antibodies adalimumab, brodalumab, infliximab, ixekizumab, and ustekinumab (Sect. 11.1).

2.2.4.3 Maculopapular Exanthema

Maculopapular exanthema, also called morbilliform drug reaction, morbilliform exanthem, and maculopapular drug eruption, is the most frequently seen pattern of drug-induced skin eruption. Clinical presentation is diverse and can vary from an erythematous rash mimicking a viral or bacterial exanthem to generalized symmetric eruptions of both isolated and confluent erythematous plaques. These often start on the trunk and then spread to the extremities and neck without involvement of the mucosa. The pink to red macules or papules tend to blanch when pressed and purple, non-blanchable spots (purpura) may appear on the lower legs. Reactions appear 7–14 days after exposure to drug but after only 1 or 2 days in already sensitized patients. Reactions usually tend to progress over a few days before regressing over a 2-week period often with accompanying desquamation. This may be the course of events even if the drug has been withdrawn. Reactions may be associated with a mild fever and itch. Histologically, lymphocytes are seen in papillary dermis and at the junction of the dermis and epidermis while degenerated, necrotic and some dyskeratotic keratinocytes and spongiosis are visible in early lesions. Maculopapular exanthema can be caused by a wide variety of drugs. Those commonly implicated are chiefly antibiotics, especially aminopenicillins (Fig. 2.7) and cephalosporins, but also sulfonamides, anticonvulsants like carbamazepine, allopurinol, and NSAIDs.

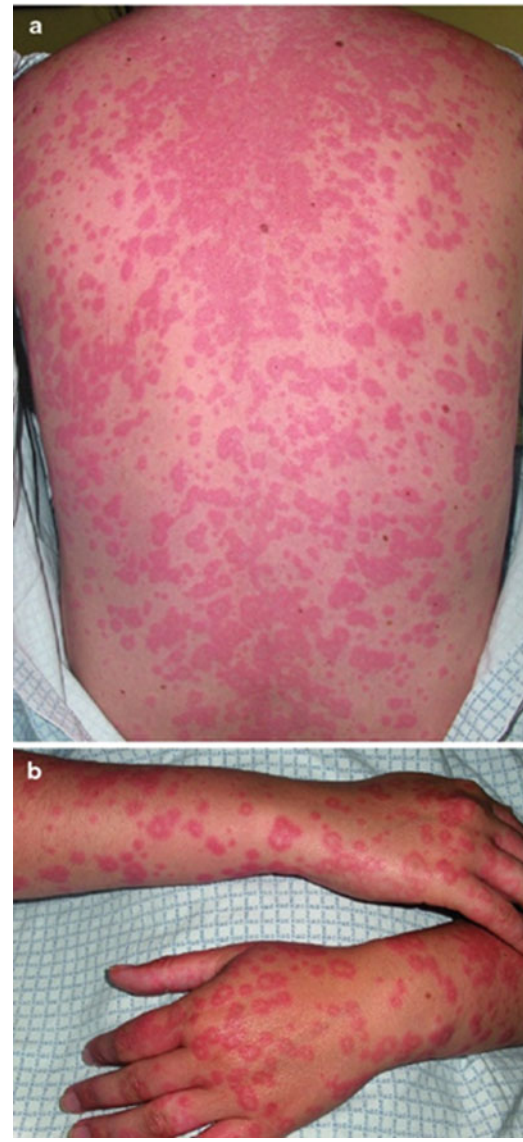

Fig. 2.7 Generalized maculopapular exanthema following the introduction of amoxicillin therapy showing lesions on the trunk (**a**) and targeted lesions on the hands and forearms (**b**). The patient had positive patch tests to amoxicillin and ampicillin and negative tests to benzylpenicillin, dicloxacillin, and a number of cephalosporins. From Gonçalo M, in: Johansen JD, Frosch PJ, Lepoittevin J-P, editors. Contact Dermatitis. 5th ed. Berlin: Springer-Verlag; 2011. With kind permission from Springer Science+Business Media

2.2.4.4 Acute Generalized Exanthematous Pustulosis

AGEP, also termed toxic pustuloderma and pustular drug eruption, is induced in 90 % of cases by drugs and characterized by fever and acute nonfollicular pustular eruptions that overlay erythrodermic skin. The incidence of the disease is said to be about three to five cases per million per year with a mortality rate of about 5 %. Reactions generally begin with a rash on the face or in armpits and groin a few days after administration of the offending drug before becoming widespread. Time of onset after drug administration can be

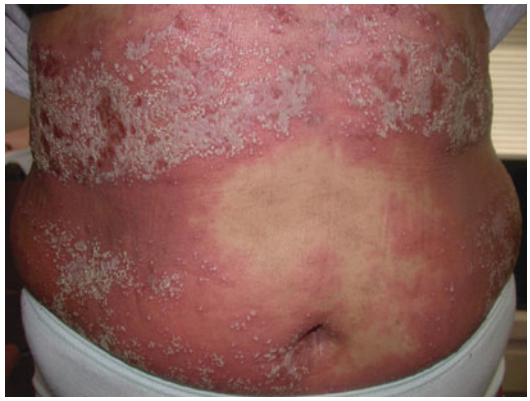

Fig. 2.8 A case of acute generalized erythematous pustulosis (AGEP) to hydroxychloroquine sulfate (photograph courtesy of Dr. Adrian Mar)

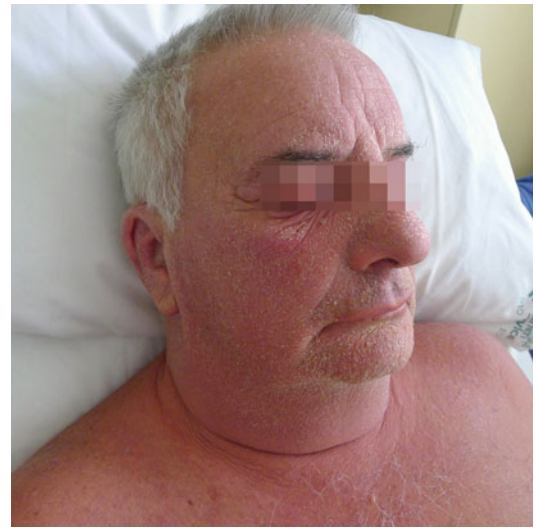

Fig. 2.9 A patient with drug reaction (or rash) with eosinophilia and systemic symptoms (DRESS), also referred to as hypersensitivity syndrome or drug-induced hypersensitivity syndrome. The patient experienced systemic symptoms, skin reactions with nonspecific maculopapular rash, and exfoliative dermatitis with facial edema (photograph courtesy of Dr. Adrian Mar)

remarkably short—even as little as 24 h in some cases. The rash lasts about 2 weeks, and the reaction regresses with skin desquamation as it resolves 5–10 days after drug withdrawal, often with rapid spontaneous healing. AGEP is characterized by symmetrical widespread edematous erythema with small non-follicular sterile pustules (Fig. 2.8) due to neutrophil accumulation predominating in body folds. Recruitment of neutrophils occurs in the late phase of development of lesions. Histopathology shows spongiosis, subcorneal pustules, leukocytoclastic vasculitis, discrete vacuolar keratinocyte degeneration, and dermal and intradermal infiltration of lymphocytes. AGEP is also characterized by the great predominance (over 80 % of cases) of antibiotics (including amoxicillin, ampicillin, tetracycline, spiramycin, pristinamycin, and metronidazole) as causative agents. Other implicated drugs include hydroxychloroquine, cotrimoxazole, diltiazem, terbinafine, and carbamazepine.

2.2.4.5 Drug Reaction (Rash) with Eosinophilia and Systemic Symptoms

Drug reaction (or rash) with eosinophilia and systemic symptoms (DRESS), also referred to as hypersensitivity syndrome or drug-induced hypersensitivity syndrome, is a severe life-threatening adverse drug reaction with an incidence reported to be between 1 in 1,000 and 1 in 10,000 depending on the drug. DRESS involves systemic symptoms and skin reactions with nonspecific maculopapular rash or a more generalized exfoliative dermatitis and facial edema (Fig. 2.9). Symptoms consist of fever, malaise, arthralgia, enlarged lymph nodes, hepatitis, renal impairment, pneumonitis, and hematological abnormalities with atypical activated lymphocytes and elevated levels of eosinophils that infiltrate the skin and other organs and are thought to cause tissue damage. Visceral involvement differentiates DRESS from other exanthematous serious acute drug reactions. Another distinguishing feature is the extended interval between drug exposure and the onset of symptoms, which is usually about 2–8 weeks. DRESS also tends to regress more slowly and the disease may sometimes reactivate after exposure to an unrelated drug or even without drug exposure. Viruses may have a role in the development of the symptoms of DRESS. This is suggested by reactivation of the Herpesviridae viruses, cytomegalovirus, EBV, and human herpes virus 6 (HHV-6), the latter evidenced by the rise of anti-HHV-6 IgG titers and the presence of HHV-6 DNA 2–3 weeks

after the onset of rash. The main drugs involved in eliciting reactions are the anticonvulsants carbamazepine, lamotrigine and phenytoin, phenobarbitone, dapsone, sulfonamides, allopurinol, minocycline, and mexiletine. In a retrospective analysis of 1,544 DRESS cases reported to the United States Food and Drug Administration between 2004 and 2010, 137 cases (8.9 %) had a fatal outcome; the sex ratio was five females to four males and the most frequently involved age range was the 60–69 group. Those older than 70 had a higher incidence of fatalities. Approximately 60 % of cases developed DRESS within 4 weeks but some late-onset cases developed after 6 months exposure to the drug. The top 20 drugs most frequently implicated in provoking DRESS, in order of highest to lowest frequency, were: carbamazepine, sulfasalazine, allopurinol, vancomycin, amoxicillin, lamotrigine, phenytoin, minocycline, zonisamide, abacavir, ciprofloxacin, piperacillin, rifampicin, ibuprofen, diclofenac, valproate, acetaminophen, phenobarbital, lamivudine, omeprazole. Increased reporting of DRESS is apparent, although this increase is not related to newly marketed drugs but rather the already well-known causes. Only 12 % of the 1,544 cases reported to the FDA were from the USA whereas 527 cases (34 %) were reported from France, 286 (19 %) from Japan, 79 (5 %) from the UK, and 135 cases (9 %) unknown. It is clear that there is underreporting to the extent, as some have claimed, that the reports received by the FDA are only 1–10 % of the real number. Finally, there is some disagreement over the variability of the clinical pattern of cutaneous symptoms and the classification of some patients as having DRESS. Some claim that use of the term DRESS in not consistent; cutaneous and systemic signs vary and eosinophilia is not a constant finding. With this in mind, the designation DIHS/DRESS to include DRESS and drug-induced hypersensitivity syndrome (DIHS) has been suggested.

2.2.4.6 Fixed Drug Eruption

Fixed drug eruption (FDE) is due to drug hypersensitivity in more than 95 % of cases. Patients may complain of burning in the affected area before the appearance of lesions but systemic symptoms are usually absent. The period required for sensitization ranges from weeks to years and the time between drug administration and eruption can be anything from a day or two to a few weeks. Initially, one or a small number of round or oval pruritic, well circumscribed, erythematous macules appear (Fig. 2.10). These may progress to edematous plaques or bulla (Fig. 2.11) and may regress over a period of 10–15 days. In a few cases, lesions can be so widespread that it is difficult to distinguish FDE from TEN. FDE is so named because the site of

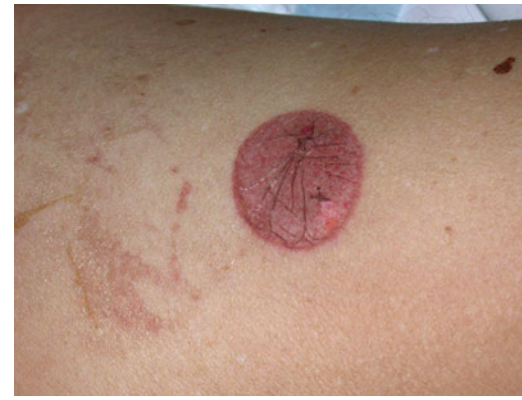

Fig. 2.10 A fixed drug eruption showing the characteristic, often-seen circular shape. Lesions often resolve with post-inflammatory pigmentation (photograph courtesy of Dr. Adrian Mar)

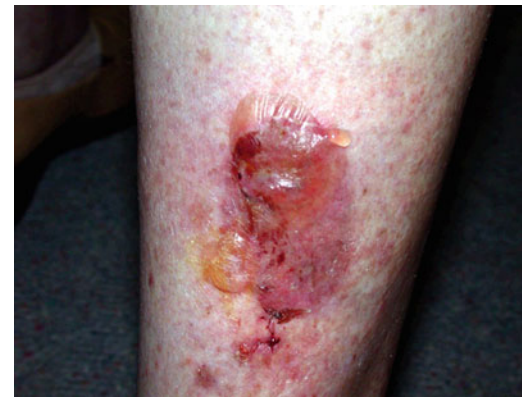

Fig. 2.11 An example of a well-circumscribed bullous fixed drug eruption. The reaction was induced by carbamazepine, a drug implicated in some severe drug-induced delayed hypersensitivity responses (photograph courtesy of Dr. Adrian Mar)

the eruption is fixed, that is, it occurs in exactly the same place when the same drug is again encountered. Upon discontinuation of the drug, lesions resolve leaving hyperpigmentation although pigmentation is often absent when the skin is fair. A FDE can occur anywhere on the skin or on mucous membranes, but the most commonly affected sites are the hands, feet, penis, groin and perianal areas, and the lips. Drugs implicated in causing FDE include allopurinol, cotrimoxazole, antibiotics (tetracycline, doxycycline, amoxicillin, ampicillin, cephalosporins, and clindamycin), NSAIDs (aspirin, ibuprofen, naproxen, and diclofenac), acetaminophen, carbamazepine, dapsone, quinine, phenolphthalein, and benzodiazepines. To confirm diagnosis of FDE, oral challenge with a single dose of the suspected drug at one-tenth the normal therapeutic dose is accepted as safe. Interestingly, desensitization has been successful for allopurinol-induced FDE but allopurinol appears to remain the only drug where desensitization for FDE has worked.

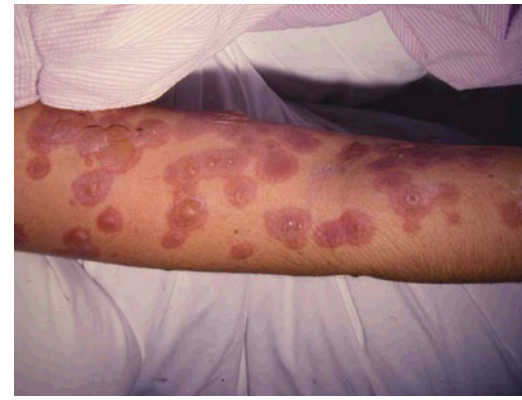

Fig. 2.12 Erythema multiforme with circumscribed macular and papular lesions progressing to plaques with dark and purpuric centers (photograph courtesy of Dr. Adrian Mar)

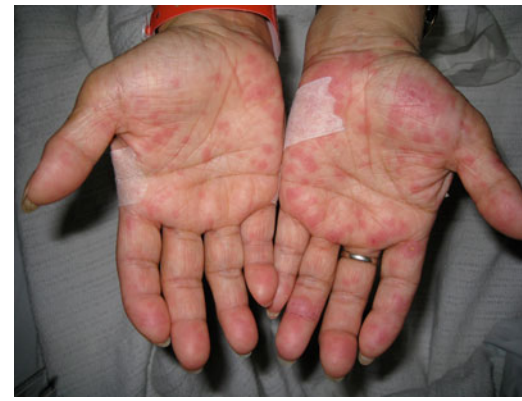

Fig. 2.13 Erythema multiforme involving the hands (photograph courtesy of Dr. Adrian Mar)

2.2.4.7 Erythema Multiforme

Erythema multiforme is a self-limiting cutaneous hypersensitivity reaction to infection (mostly) or drugs occurring mainly in adults 20–40 years of age although it can occur in patients at any age. Prodromal symptoms are either lacking or mild (itch, burning) and the condition usually resolves spontaneously in 3–5 weeks without sequelae. Although considered to be closely related to SJS and TEN, and with the designation erythema multiforme major sometimes interchanged with the former, erythema multiforme is now accepted as a distinct condition. Major reasons for this are its lesser severity, minimal involvement with mucous membranes, and the extent of epidermal detachment which is usually less than 10 %. Lesions appear as circumscribed pink-red macules before progressing to papules which may enlarge into plaques, the centers of which become darker or even purpuric (Fig. 2.12). Crusting or blistering may occur. Several days after onset, lesions of various morphology are visible, hence the name "multiforme." Palms (Fig. 2.13) and soles may be affected and if the mucosa is involved, it is usually limited to the oral cavity. Patients may experience multiple outbreaks in a year. More than half the cases of erythema multiforme are due to *Herpes simplex* (HSV), and recurrent cases may be secondary to reactivation of HSV-1 and HSV-2. Acyclovir, famciclovir, or valacyclovir have been used to treat recurrent outbreaks. *Mycoplasma pneumoniae* and fungal infection are also commonly involved with reactions. Drugs most commonly associated with erythema multiforme are anticonvulsants, barbiturates, ciprofloxacin, NSAIDs, penicillins, phenothiazines, sulfonamides, and tetracyclines. Some drugs released in more recent years are now being implicated, for example, the monoclo-

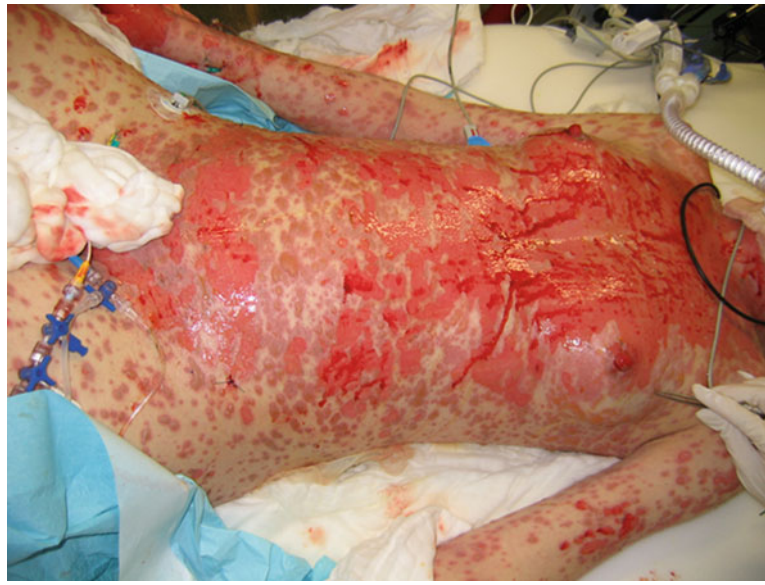

Fig. 2.14 Toxic epidermal necrolysis in an adult patient covering more than 30 % of the total body surface area. From Struck MF et al. Severe cutaneous adverse reactions: emergency approach to non-burn epidermolytic syndromes. Intensive Care Medicine 2010;36:22. With kind permission from Springer Science+Business Media

nal antibody adalimumab, bupropion, candesartan cilexetil, and metformin and reactions to some vaccines against bacteria (diphtheria–tetanus) and viruses (hepatitis B, hepatitis C, cytomegalovirus, and HIV) are seen.

2.2.4.8 Stevens–Johnson Syndrome and Toxic Epidermal Necrolysis

SJS and TEN are potentially fatal, severe, rare, adverse cutaneous drug reactions involving both the skin and mucous membranes. Beginning with relatively unremarkable signs like fever, malaise, and perhaps eye discomfort, macular lesions become symmetrically distributed on the trunk, face, palms, and soles. Lesions develop a central bulla and coalesce into large sheets of necrotic tissue covering at least 30 % of the body in the case of TEN (Fig. 2.14). For SJS, skin denudation/detachment is generally less than 10 % of body surface area, and this extent of skin involvement is the main distinguishing feature between the two diseases. Skin erythema and erosions progress to the extremities with more than 90 % of patients experiencing ocular, buccal, and genital mucosa involvement. Figure 2.15 shows lip and facial involvement in a child with developing drug-induced SJS. Respiratory and gastrointestinal tracts can also be affected. Ocular effects can include acute conjunctivitis with discharge, eyelid edema, erythema, and corneal erosion or ulceration. The collective clinical features sometimes do not fit neatly into

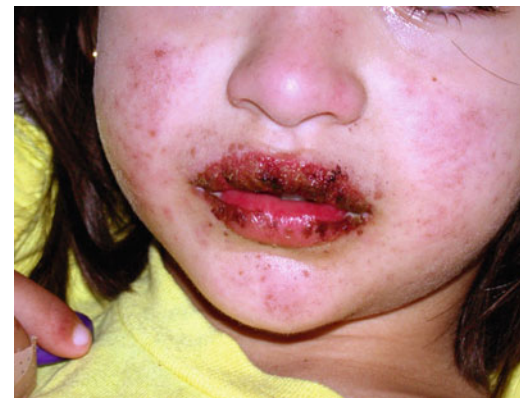

Fig. 2.15 Lips and facial involvement in a child with developing drug-induced Stevens–Johnson syndrome (photograph courtesy of Dr. Adrian Mar)

either a clear SJS or TEN diagnosis and may be classified as overlapping SJS or TEN. In the SJS–TEN overlap group, primary lesions tend to be dusky red or purpuric macules or flat atypical targets widely distributed as isolated lesions but with quite marked confluence on the face and upper trunk. As with TEN, but not necessarily with SJS, systemic symptoms are always present. Skin detachment occurs on 10–30 % of the body surface area.

In the second phase of development of the diseases, large areas of epidermis become detached. Sequelae and late effects are common in SJS and particularly TEN. These can include changes in skin pigmentation, nail dystrophies, and ocular problems. Long-term sequelae in surviving TEN patients can include eye afflictions like entropion and symblepharon, on-going mucous membrane erosion, and cutaneous scarring. Dry mouth and dry eyes resembling Sjögren syndrome may be a long-term complication.

Although SJS and TEN are rare with incidences of usually less than two per million per year (e.g., 1.89 Western Germany, 1.9 USA), some infectious diseases can have a marked effect in the incidence. This is seen with HIV where the annual incidence is approximately 1,000 times higher than in the general population. Infections *M. pneumoniae* and *H. simplex* are known to cause SJS and TEN without drug involvement. A strong association shown between HLA-B*15:02, SJS, and carbamazepine in Han Chinese and confirmed in a Thai population is a clear demonstration of a relationship between ethnicity and drug hypersensitivity. This finding has stimulated interest in the investigation of genetic factors in drug hypersensitivity (see Sect. 3.4); one recent demonstration being the large collaborative European RegiSCAR project which showed that HLA-B*15:02 is not a genetic marker for carbamazepine, sulfamethoxazole, lamotrigine, or NSAIDs of NSAID oxicam-type-induced SJS/TEN in Europe. The drugs most commonly responsible for SJS/TEN have been divided by Roujeau into those that provoke the disease after only a short period of administration and those that do so after being taken for longer periods. In the short period group are trimethoprim–sulfamethoxazole, other sulfonamides, aminopenicillins, cephalosporins, quinolones, and chlormezanone. In the long period group: carbamazepine, phenytoin, phenobarbitone, and valproic acid, NSAIDs of the oxicam type, allopurinol, and corticosteroids. Allopurinol is the drug most commonly implicated in causing SJS/TEN in Europe and Israel. Some newer drugs identified as being of increased comparative risk include nevirapine, lamotrigine, and sertraline. Because of the fear of provoking a second episode or aggravating an existing case of SJS/TEN, skin testing in all its forms and provocation tests are not to be considered although there are at least two reports of intradermal testing that did not trigger another episode. Patch testing and the lymphocyte transformation test (Sect. 4.7.1) have been used but proved to be of low sensitivity. Clinical features and histology therefore remain the mainstay of diagnosing SJS/TEN.

Summary

- In the Gell and Coombs classification of allergic reactions, four types of hypersensitivities designated types I, II, III, and IV are distinguished.
- Type I, also called immediate or anaphylactic hypersensitivity, occurs within about 30 min but reactions can be dramatic and appear within seconds or minutes as in anaphylaxis.
- Type I reactions are IgE antibody-mediated. Receptor-bound drug-reactive IgE on the surface of mast cells is cross-linked by complementary drug determinants causing cell degranulation and the release of inflammatory mediators.
- Drugs well known to cause type I reactions include penicillins, cephalosporins, neuromuscular blocking drugs, some NSAIDs, monoclonal antibodies, quinolones, and proton pump inhibitors.
- The term “anaphylactic” is used to describe an immune-mediated immediate systemic reaction involving the release of potent mediators from mast cells and basophils that produce

a range of possible symptoms including erythema, urticaria, angioedema, bronchospasm, gastrointestinal symptoms, and cardiovascular collapse. The term "anaphylactoid" is used for reactions that show clinically similar signs and symptoms but where no immune-mediated mechanism can be demonstrated.

- It is important to have a suitable clinical grading system for anaphylaxis.
- Urticaria is the second most common cutaneous reaction induced by drugs, often in association with angioedema and anaphylaxis. Many drugs are implicated including β-lactams, NSAIDs, sulfonamides, vancomycin, and contrast media. ACE inhibitors are responsible for approximately one in six patients admitted to hospital with angioedema.
- Type II hypersensitivity is also known as antibody-dependent cytotoxic hypersensitivity. Drugs can attach to cell membranes producing drug-induced hemolytic anemia, thrombocytopenia, and granulocytopenia. Drugs implicated: hemolytic anemia—penicillins, quinidine, methyldopa; thrombocytopenia—quinine, quinidine, propyl thiouracil, vancomycin, sulfonamides; granulocytopenia—pyrazolones, thiouracil, anticonvulsants, and sulfonamides.
- Type III hypersensitivity is mediated by soluble immune complexes mostly involving IgG antibodies. Drug-induced serum sickness-like reaction is the prototype example of type III drug hypersensitivity. Hypersensitivity vasculitis is another example of a type III hypersensitivity response induced by drugs.
- Type IV hypersensitivity reactions are mediated by antigen-specific effector T cells. Reactions generally occur 48–72 h after antigen exposure and are therefore referred to as delayed reactions.
- Important delayed cutaneous reactions include maculopapular exanthema; allergic contact dermatitis; psoriasis; acute generalized exanthematous pustulosis; drug reaction with eosinophilia and systemic symptoms; fixed drug eruption; erythema multiforme; Stevens–Johnson syndrome; and toxic epidermal necrolysis.

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Mechanisms of Hypersensitivity

3

Abstract

Allergic reactions to drugs are not always the result of the drug's protein-binding capacity, biotransformation, or degradation. Mediator release may occur via cross-linking of cell-bound IgE by di-(multi-) valent free drug. Physiological and pharmacological effects of histamine are mediated through four receptors, H_1, H_2, H_3, and H_4. The H_3 receptor has a regulatory role in the release of neurotransmitters such as serotonin and dopamine; the H_4 receptor exerts a chemotactic effect on several cell types associated with allergy and asthma. Cysteinyl leukotrienes and PAF are powerful mediators of anaphylaxis, asthma, and shock. Sphingosine-1-phosphate, elevated in the lungs of asthmatics, regulates pulmonary epithelium permeability and contributes to the pathogenesis of anaphylaxis. Urticaria is a heterogeneous disease with many subtypes. Both ACE inhibitors and angiotensin II receptor blockers may cause angioedema. Abacavir changes the shape of the HLA antigen-binding cleft producing an alteration in the repertoire of self-peptides that bind HLA-B*57:01 and a T cell response to self-proteins. Drug-induced delayed-type cutaneous hypersensitivity reactions are mediated by CD4+ and CD8+ CD3+ T cells in the dermis and epidermis. Granulysin appears to be a key molecule for keratinocyte killing in TEN/SJS. Drugs provide good examples of types II (immune hemolytic anemia, drug-induced thrombocytopenia) and III (serum sickness-like) hypersensitivities.

In this chapter, emphasis has been placed on the core mechanisms underlying the broad categories of hypersensitivity responses distinguished on the basis of the Gell and Coombs classification and based on differences in the immune reactants (antibodies or cells), the form of the presented antigen, and the effector mechanisms involved. Mechanisms involved in individual drug hypersensitivities including, for example, responses to reactive metabolites from chemically "inert" parent drugs such as sulfamethoxazole; relationships between chemical structures and immune responses seen with, for example, anaphylactic reactions to neuromuscular blocking drugs during anesthesia; hypersensitivities and other intolerances to nonsteroidal anti-inflammatory

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_3, © Springer Science+Business Media, LLC 2013

drugs (NSAIDs); and mechanisms underlying the killing of malignant cells by some drugs used in chemotherapy are not confined to this chapter but presented in the relevant chapters dealing with pharmacologically different groups of drugs. Most hypersensitivities to drugs manifest as type I or type IV reactions. Type II and type III drug hypersensitive reactions are far less often seen and are considered after the discussions of the type I and IV responses. Mechanisms, to the extent that they are currently understood, of other types of "hypersensitivity" reactions or intolerances, some mediated by antibodies other than IgE, and others by cells, are also discussed. We begin by examining the mechanisms underlying type I drug-induced IgE antibody allergic sensitization, regulation and production, and the effector mechanisms operative in IgE-mediated allergic reactions.

3.1 Allergic Sensitization to Drugs and the Dogma of Previous Exposure

3.1.1 Immunogenicity of Free and Conjugated Drugs

As well as the chemical nature of a drug, its size and complexity influence its antigenicity. Chemicals of molecular mass less than 5 kDa and sometimes up to about 10 kDa are often poorly or non-antigenic. From the time of the early immunochemical studies on antigenicity and haptens, organic chemicals of small molecular mass have been assumed to be antigenic and capable of stimulating an immune response only as a complex with a macromolecular carrier, usually protein. By coupling a wide range of different chemicals that are not antigenic in their free state, for example, steroids, sugars, purines, pyrimidines, nucleosides, and aromatic ring compounds such as phenols, etc., Landsteiner and other early investigators demonstrated clear and specific antibody responses in laboratory animals. Chemicals such as drugs may form hapten–carrier complexes in vivo in three different ways—by direct chemical covalent interaction with a soluble or cell-bound protein, by biotransformation of the drug to form a reactive metabolite able to bind to a carrier protein, or by degradative changes to the parent molecule forming reactive groupings (Fig. 3.1). In practice, however, it is often not possible to show protein binding by a drug or to even offer a satisfying explanation of how such binding might occur given the known chemical properties of the drug and the metabolic processes to which it is exposed. While a number of allergenic drugs such as the β-lactams undergo well-known ring-opening and subsequent protein-binding reactions (Chap. 5), for many drugs chemical reactivity, protein binding, biotransformation, or the involvement of degradative products an*d*/or reactive impurities has not been demonstrated. This raises the question of another possible mechanism(s) to explain the immune recognition of "small" drug molecules and the subsequent immunological steps involved in the drug-induced hypersensitivity responses (see Sects. 3.1.2, 3.1.3, 3.4, 6.2.3.3, 7.4.2.3, and 7.4.5.3). Over the last few decades, a large number of drugs have been implicated as provoking agents for a range of hypersensitivity states, and although the list of identified drug allergenic determinants has expanded, this aspect of hypersensitivity research is still in its infancy. Structures as diverse as substituted ammonium ions, simple disaccharides, small side chain groups and ring structures on β-lactams, halogenated isoxazolyl groups, and whole molecules as seen with trimethoprim and chlorhexidine are known to be recognized as allergenic determinants in some drug-allergic patients. Of the presently known drug allergenic determinants, most have been reliably identified in IgE antibody recognition studies using quantitative immunochemical hapten inhibition techniques. The approaches and methods already applied so successfully to a range of drugs (see Chaps. 5–8) need to be expanded to cover other yet-to-be-defined IgE antibody-binding determinants and extended to T cell-mediated drug hypersensitivity reactions where progress has been slow. Results from the IgE studies have demonstrated that more than one allergenic determinant generally occurs on drugs and such antigenic

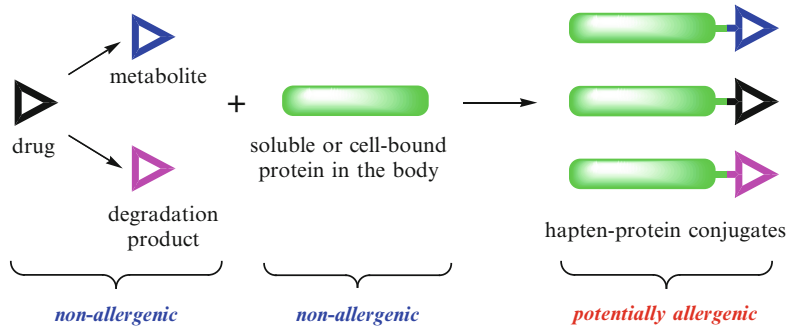


Fig. 3.1 Diagrammatic representation of possible, and potentially allergenic, hapten–protein complexes that may form in vivo from a drug and/or its metabolite(s) and degradative product(s). From Baldo BA & Pham NH. Structure–activity studies on drug-induced anaphylactic reactions. Chem Res Toxicol 1994; 7: 703. Reproduced with permission from American Chemical Society

heterogeneity is reflected in patients' IgE antibody recognition responses. As more drug allergies and more allergic individuals are studied, the extent of this heterogeneity will emerge and with it the possibility of gaining greater insights into the structural basis of drug allergenicity.

For an allergic reaction to a given drug, immunological dogma requires that the response occurs on reexposure to the drug *after* the initial sensitizing exposure to that drug. However, this seemingly obvious requirement may not always hold true or appear to hold true. Some allergic responses, sometimes even life-threatening as with anaphylaxis, occur on first exposure to a drug. Such reactions to the neuromuscular blocking drugs are well known and there are numerous other investigations and case studies involving a variety of pharmacologically different drugs including trimethoprim, iodinated contrast media, opioids, and some antibiotics that report the same phenomenon. In some cases, this might be explained by previous exposure to a structurally similar drug or to a structurally similar compound that may not even be administered as a drug. An example of the former case is a reaction to a cephalosporin in a patient previously given a penicillin while a reaction to a drug may also result from previous exposure to the drug (e.g., an antibiotic in meat) or an antigenically cross-reactive chemical in some foods or in the environment. Although IgE antibodies are almost invariably thought of as induced humoral responses to allergens, parasites, and fungi, some of the antibodies are "natural," that is, antibodies formed without exposure to foreign antigens via infection or passive or active immunization. Examples of such antibodies appear to be those that are complementary to various cross-reactive carbohydrate determinants (the so-called CCDs), and to phosphorylcholine connected by phosphodiester linkages in some *N*-linked proteoglycans and glycolipids and found in pneumococcal teichoic acid ("C substance") and other "C substances" in bacteria, fungi, arthropods, helminthes, protozoa, and plants. The curious connection between IgE natural antibodies to the D-galactose disaccharide found on cetuximab, a chimeric mouse–human IgG_1 monoclonal antibody used for cancer treatment, and anaphylaxis in some treated patients (see Sect. 11.1.3.2) and the possible cross-reaction of natural anti-phosphorylcholine IgE antibodies with ammonium groups on neuromuscular blocking drugs (Sect. 7.4.5.3) are indicators of the likely existence of other natural IgE antibodies with potentially cross-reactive specificities. Although some of these antibodies may appear to have no connection whatsoever with a particular drug, structural features recognized by the antibody combining site may resemble structures on the drug molecule resulting in allergenic cross-reactivity. It should also be kept in mind, however, that not all exposures to a potentially sensitizing drug will result in a patient becoming

sensitized and every sensitized patient will not necessarily respond with allergic symptoms following reexposure to the sensitizing drug.

Findings so far on immune recognition of drugs, especially recognition by IgE antibodies in cases of type I immediate hypersensitivity and to a much lesser extent for drug recognition by specific T cells, have shown that small parts of drug molecules, sometimes only one or a few chemical groups that form part of the molecule, constitute the allergenic determinant structures that are complementary to the immunoglobulin E combining sites and T cell receptors. In addition to allergic cross-sensitivity to drugs in the same family, for example, between different β-lactams, recognition of widely distributed structures commonly occurring in many different drugs and chemicals also represents potentially immunologically cross-reactive determinants. Substituted ammonium ions identified as the most important IgE antibody-binding structures in neuromuscular blocking drugs (Sect. 7.4.2.1) occur in many drugs and chemicals frequently encountered by humans. Bioisosteres, that is, groups with similar physical and/or chemical properties that impart similar biological properties to a drug, should also be kept in mind when prior allergic sensitization is suspected in patients who are first time reactors to a drug. In this era of so-called rational drug design, bioisosteres are commonly seen for example, in the replacement of a six-membered phenyl ring with a five-membered thiophene ring in many synthesized drugs. The importance of bioisosterism in the identification of allergenic structures and allergic cross-reactivity is discussed further in Chap. 5.

3.1.2 Mediator Release by Free and Conjugated Drugs in Immediate Allergic Reactions

In allergic subjects, IgE antibodies, as well as being free in serum, are fixed to the extracellular D1 distil and D2 proximal domains of the FcεRI receptor on mast cells and basophils via the Cε2 and Cε3 domains of the antibody Fc region. Bridging of adjacent cell-bound IgE molecules by at least bivalent allergenic determinants reacting with their complementary antibody combining sites (Fig. 3.2a–d) triggers cell degranulation and release of a variety of mediators that cause the signs and symptoms of a type I hypersensitivity reaction. In the case of drug–carrier conjugates, cross-linking of IgE antibodies is readily explained by the presence on the conjugates of multiple reactive drug determinant sites, but for free, uncomplexed drug molecules both the size and number of reactive determinants would appear to be too small for cross-linkage of antibody combining sites to occur. Drugs with a single IgE-binding determinant cannot, of course, cross-link adjacent cell-bound antibody molecules (Fig. 3.2e), but even if two or more determinants are present, they must be separated by a suitable distance and/or be suitably spatially arranged if cross-linking via adjacent complementary antibody combining sites is to occur (Fig. 3.2f, g). Despite this problem of explaining the mechanism of apparently monovalent drug-induced allergic mediator release, there is at least one group of drugs, the neuromuscular blockers (and probably more to be identified) that can specifically elicit antibody-induced mast cell activation and release without first undergoing coupling to a macromolecular carrier. For these drugs, di- or multi-valency is an inherent part of the molecular structure and, even in the absence of protein binding, cross-linking of cell-bound antibodies can be effected (Fig. 3.2a). Of the polymethylene bismethonium compounds, the 2 nm molecular length of the C-10 congener decamethonium is optimal for neuromuscular block, that is, it best fits the distance between the receptive sites on the muscle nicotinic acetylcholine receptor. Interonium distances, however, are less, for example, 1.4 nm for decamethonium, 1.08 nm for *d*-tubocurarine (molecular length 1.8 nm), and 1.14 nm for pancuronium (molecular length 1.9 nm). These distances appear to be suitable for the neuromuscular blockers to bridge and thus activate adjacent IgE molecules on the mast cell surface (see also Sects. 7.4.2.1 and 7.4.2.3).

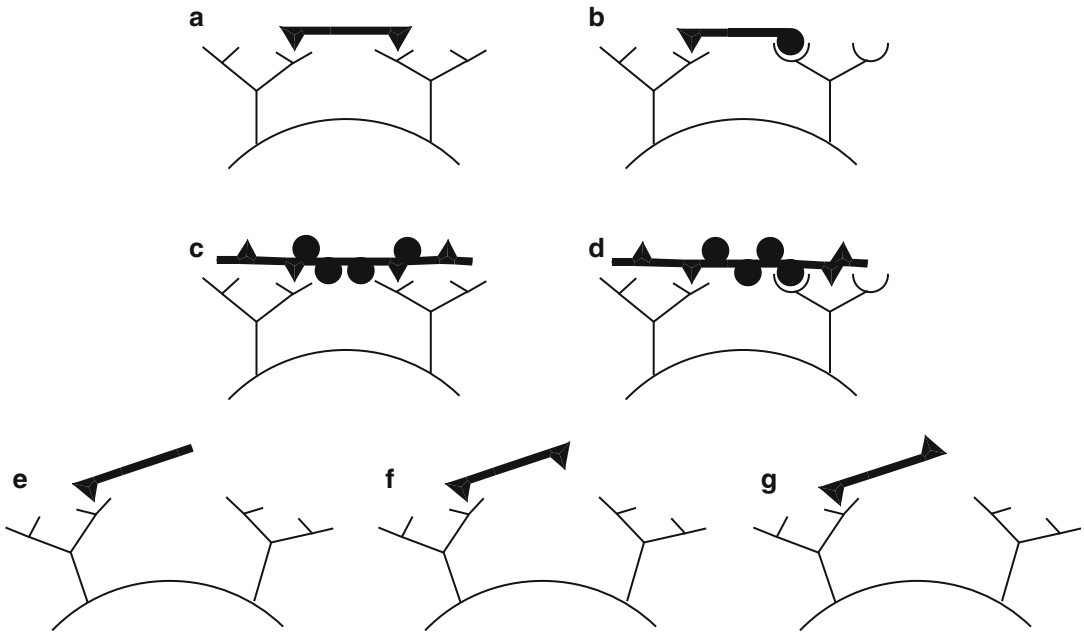


Fig. 3.2 Different ways in which a free drug (*shown in bold* in **a**, **b**, **e**, **f**, and **g**) and a drug–protein conjugate (**c**, **d**) may cross-link or bridge adjacent cell-bound IgE molecules which triggers release of the mediators of immediate hypersensitivity. (**a**) Bridging via an allergenically divalent unconjugated drug molecule with the same or closely related allergenic determinants. This is the mechanism thought to occur in patients who experience anaphylaxis following administration of a neuromuscular blocking drug. (**b**) Bridging via a free, unconjugated drug molecule containing two (or more) different determinants that elicit an IgE response. (**c**) and (**d**) Bridging via conjugated drug molecules with cross-linking effected by the same, or different, determinants, respectively. Failure to bridge adjacent cell-bound IgE molecules because: (**e**) drug is allergenically monovalent; (**f**) and (**g**) drug determinants are not positioned to effect cross-linkage. From Baldo BA & Pham NH. Structure–activity studies on drug-induced anaphylactic reactions. Chem Res Toxicol 1994; 7: 703. Adapted with permission from American Chemical Society

3.1.3 Immunological Recognition of Free, Unconjugated Drug Molecules

The generally accepted explanation for the recognition of drugs causing an immune-mediated hypersensitivity reaction is based on the binding of drug to a protein carrier molecule, immune recognition and processing of the drug–protein complex, presentation of drug–peptide conjugates to the T cells, and recognition and reaction of the T cell with the drug antigen. However, although there is no evidence that many drugs, either as the parent compound or as a metabolite, bind to a suitable carrier, there is evidence that T cells recognize metal ions such as Ni^{2+} and some drugs like sodium aurothiomalate that do not require antigen processing. In one explanation, the drug is said to bind directly to self-peptides in the antigen-binding cleft of the major histocompatibility complex (MHC). In another possible alternative, the drug may couple directly to the MHC itself on regions involved in binding to the T cell receptor. In drug interaction with the MHC, recognition may be restricted to a limited number of peptides or it may be promiscuous, that is, independent of peptide. For some drugs at least, direct stimulation of T cells via the T cell receptor in an MHC-dependent way has been suggested. With sulfamethoxazole for example, a drug known to be metabolized to its reactive nitroso derivative, only a minority of T cell clones reactive with this metabolite were isolated from sulfamethoxazole-allergic patients. The short time period for T cell activation to occur with some free, unmetabolized drugs, T cell clone

reactivity with glutaraldehyde-fixed antigen-presenting cells, and removal of free drug by washing all suggests a drug–T cell receptor interaction that is independent of metabolism and processing. Further consideration of the recognition and the immune response to free, unconjugated drugs is set out in Sect. 3.4 below.

3.2 IgE Antibodies and IgE-Mediated Drug Hypersensitivities

The central importance of IgE antibodies in both the immediate and late phases of an allergic response involving inflammatory reactions is well established. IgE mediates the allergic inflammatory response by binding to both its high-affinity receptor FcεRI on mast cells and basophils and its low-affinity IgE receptor FcεRII (CD23) (on a number of different hematopoietic cells including B cells) to augment humoral and cellular responses.

3.2.1 Initiating Events in the Production of IgE Antibody

IgE is produced by plasma cells at the site of an allergic reaction generally in mucosal, cutaneous, and gut lymphoid tissue. IgE antibody production begins with the interaction between antigen-bearing antigen-presenting cells (APC) and lymphocytes. APCs can be dendritic cells, the most important cell in initiating the adaptive immune response, macrophages, and B cells. Naïve T lymphocytes not only need to have antigen presented in a special way, they also require precise signals to become activated. Both of these requirements are fulfilled by the APC firstly via the membrane-associated MHC that interacts with the T cell receptor (TCR) (activation signal 1) and secondly by the provision of co-stimulatory signals in the form of the membrane protein ligand CD80 (B7-1) working in tandem with another membrane ligand CD86 (B7-2) (activation signal 2). These ligands interact with their complementary receptor CD28 constitutively expressed on naïve T cells to allow the cells to undergo clonal expansion (Fig. 3.3). Resting or naïve B cells are also nondividing, and to undergo clonal expansion and differentiation to effector B cells, that is, to produce allergen-reactive IgE antibodies, B cells also require the participation of a specific receptor, the B cell receptor or BCR, and co-stimulation from T helper cells. The BCR has immunoglobulin anchored in the cell membrane, and, in concert with the B cell co-receptor complex, it is the interaction between this surface immunoglobulin and its complementary antigen that initiates B cell activation. Upon binding to the antigen, the BCR–antigen complex is internalized within an endosome, processing follows, and the processed antigen is presented back on the surface by MHC type II molecules. If maturation of the B cell to a plasma cell or a memory cell is to continue, interaction with, and co-stimulation by, an activated T helper cell is required. Interaction between the B cell and an activated Th2 cell with the appropriate TCR involves recognition of the MHC-processed antigen by the TCR and co-stimulatory CD80/CD86 (B7) signals. Co-stimulation of the B cell that eventually leads to clonal expansion and isotype switching is also enabled through upregulation of the CD40 ligand (CD40L) (Fig. 3.3). If CD40L-CD40 receptor interaction and co-stimulation do not eventuate, B cells undergo apoptosis and are eliminated. Cell proliferation and isotype switching for the synthesis of IgE are aided by the cytokines Il-4 and IL-13 generated by Th2 cells. These two cytokines initiate transcription of germ-line mRNA for IgE antibodies and are regarded as the first of two signals necessary for class switching from IgM- to IgE-bearing cells. The second signal is delivered by the interaction of CD40L on the T cell surface with its receptor CD40 on the B cell. This interaction results in all of the elements necessary for the ε-heavy chain being brought into close proximity.

IgE levels influence IgE receptor density on cells. High levels of antibody increase both the number of FcεRI receptors and the degranulation of mast cells and basophils. Along with degranulation, increased release of cytokines such as IL-4 occurs and these in turn stimulate increased IgE levels and receptor density. A reduction of IgE results in a reduction of receptor levels on mast

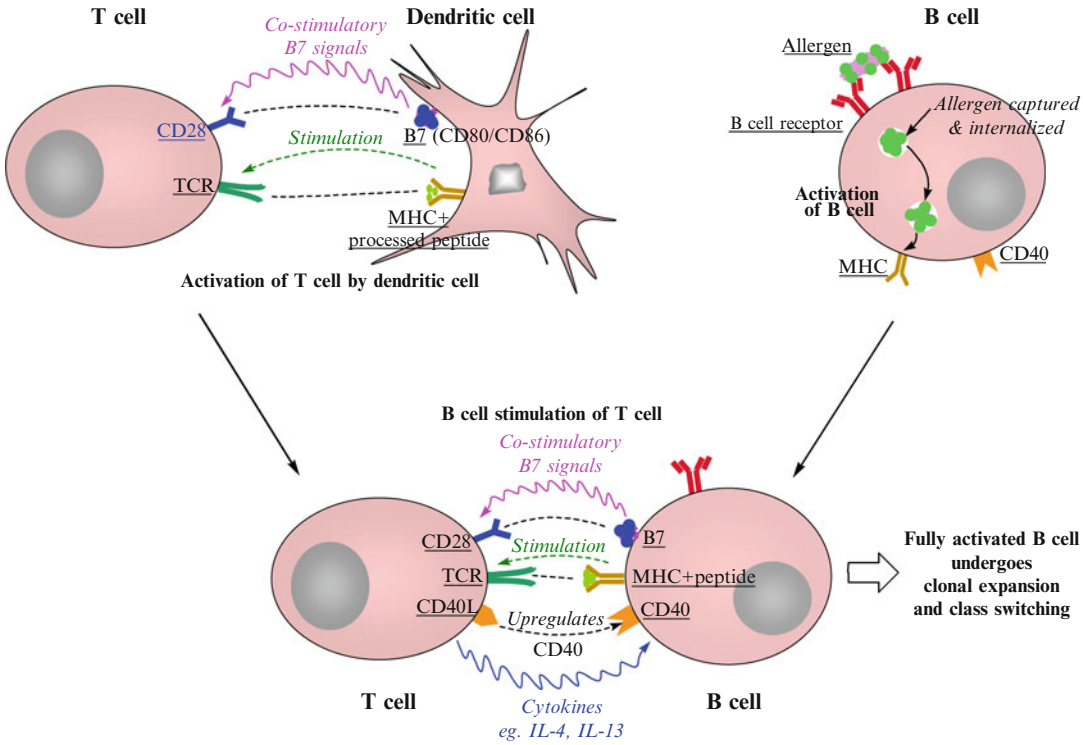


Fig. 3.3 Cellular events in the production of IgE antibodies. Presentation of antigen (usually in peptide form) to T cells via MHC molecules on dendritic cells. This results in the T cells undergoing clonal expansion. Antigen presentation to activated T cells by activated B cells ultimately results in co-stimulation of the B cells, class switching, clonal expansion, and differentiation to effector cells

cells and decreased degranulation. IgE is also capable of upregulating the FcεRII receptor (see below). From the therapeutic aspect then, inhibition of IgE is desirable since it leads to a decrease in the release of mediators from mast cells and basophils. This is, in fact, the rationale for the use of omalizumab, a recombinant humanized IgG1k monoclonal antibody (see Sect. 11.1.3.1) used for patients with difficult-to-manage severe persistent allergic asthma. Omalizumab binds to the Cε3 region of circulating human IgE antibodies inhibiting their binding to the FcεRI and FcεRII receptors and thus ultimately suppressing IgE-mediated mast cell activation and the allergic inflammatory response. It does not target receptor-bound IgE on mast cells and thus does not trigger mast cell degranulation. Another potential therapeutic approach to treat allergic disorders is the interference with the interaction between IL-4 and its receptor. Without this interaction, B cells do not differentiate into IgE-secreting plasma cells and Th2 cells and their functions in the allergic response are inhibited. Modulation of cytokines involved in the production of IgE is yet another therapeutic strategy. For example, IL-12 and IFN-γ inhibit cytokine production by Th2 cells so interference with the expression of these cytokines suppresses IgE synthesis. For further biologic strategies in directing therapies for hypersensitivities, see Chap. 11.

3.2.2 Allergic Release of Mediators of Hypersensitivity from Mast Cells

The critical role of IgE in both the immediate and late phases of the allergic response is well established and, together with the mast cell, the resultant humoral and cellular interactions produce

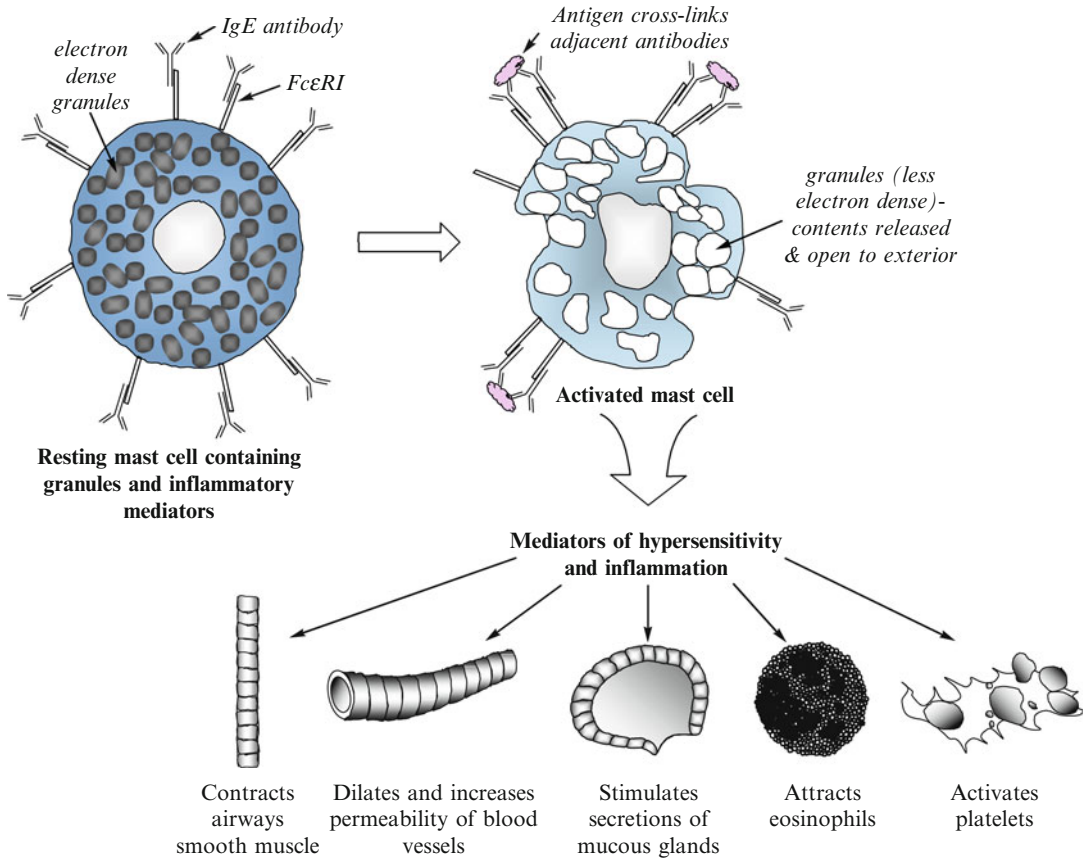


Fig. 3.4 Diagrammatic representation of antigen-induced degranulation of, and mediator release from, mast cells by antigen-effected cross-linking of adjacent cell-bound complementary IgE antibodies

the inflammatory mediators and symptoms characteristic of allergic reactions. On the basis of the type of proteases and proteoglycans in their granules, human mast cells can be divided into three populations: tryptase-only positive mast cells in the lungs and intestinal mucosa; tryptase, chymase, and carboxypeptidase positive mast cells in the skin, connective tissues, and intestinal mucosa; and a smaller population of chymase-only positive cells in the nasal and intestinal mucosae. For more details of tryptase and its importance as a diagnostic marker for anaphylaxis, the reader is referred to Sect. 4.5.1. The initial event in the activation of mast cells for mediator release is the binding of IgE antibodies to the high-affinity FcεRI IgE receptor abundantly expressed on the mast cell and basophil surfaces (Fig. 3.4). The high affinity of the receptor (~K_a 10^{-10} M) means that a high proportion of IgE is bound even in situations where there are low levels of circulating IgE antibodies. The FcεRI complex is a receptor in tetramer form made up of a ligand-binding α chain structurally related to the α chains of FcγR, a tetraspan β chain, and the FcγR γ chain dimer. The α chain has two protruding Ig type domains that bind the Cε3 region of IgE and in the presence of the antibody the receptor is upregulated while the Fc receptor for IgG is downregulated. The β and γ chains each contain an ITAM (Immunoreceptor Tyrosine-based Activation Motif) that interact with the Lyn, Syk, and Fyn protein tyrosine kinases. The critical event and signal for mediator release, as occurs in anaphylaxis, is the cross-linking

of receptor-bound IgE antibodies by allergen molecules reacting with the bivalent antibody combining sites. The IgE–FcεRI complex is long-lasting and dissociates exceptionally slowly. Cross-linking of receptors causes their aggregation, rapid migration to lipid rafts, activation of the Lyn and Fyn protein tyrosine kinases, and ultimately transphosphorylation of the β and γ chains and involvement of the Syk kinase. Mast cell degranulation (Fig. 3.4), which can occur within seconds, follows a series of activation steps induced by phosphorylation reactions discussed in more detail in Sect. 3.2.6. Some of the released mediators of inflammation and anaphylaxis stored in the cytoplasmic granules including histamine (see below, Sect. 3.2.5.1), heparin, platelet-activating factor (PAF) (Sect. 3.2.5.3), serotonin, the enzymes tryptase, chymase, and carboxypeptidase, and eosinophil, neutrophil, and monocyte chemotactic factors are preformed while others are newly synthesized. The preformed mediators are responsible for the immediate signs and symptoms of vasodilation, edema, bronchoconstriction, and itching. The newly synthesized group of released mediators includes prostaglandin D_2 (PGD_2), thromboxanes, and leukotrienes LTB_4, LTC_4, and LTD_4 (Sect. 3.2.5.2). A host of cytokines (pro- and anti-inflammatory), chemokines, and chemotactic, stimulating, and growth factors including interleukins -1, -3, -4, -5, -6, -8, -9, -10, -11, and -13, tumor necrosis factor (TNF), granulocyte–macrophage colony-stimulating factor (GM-CSF), monocyte chemotactic protein-1 (MCP-1), regulated upon activation normal T cell expressed and secreted (RANTES; CCL5), and eotaxin (CCL-11) are also released.

3.2.3 Amplification of IgE Antibody Production by Cellular Interaction

Mast cells, basophils, and even dendritic cells can accentuate B cell production of IgE antibodies by direct interaction (Fig. 3.5). IgE antibodies newly synthesized by plasma cells bind to the FcεRI receptors on the surfaces of mast cells and basophils. Cross-linkage of antibodies by antigen that reacts with the combining sites of adjacent receptor-bound IgE molecules activates the receptors and triggers the cells to express CD40 ligand (CD40L) and secrete IL-4. These molecules react with their complementary receptors expressed on the B cell surface, and hence, like Th2 cells, mast cells and basophils can induce class switching and increase the production of IgE antibody.

3.2.4 Low-Affinity IgE Receptor FcεRII (CD23)

A second receptor for IgE, the low-affinity receptor FcεRII also known as CD23, is expressed on airways smooth muscle cells and several types of hematopoietic cells including mature B lymphocytes, macrophages, monocytes, dendritic cells, and eosinophils. The designation "low" affinity is derived from the receptor's lower affinity ($K_D = \sim 10^{-7}$–10^{-8}) than the affinity of the FcεRI receptor ($K_D = \sim 10^{-10}$–10^{-11}). CD23 has multiple functions by virtue of its capacity to bind a range of different ligands. As well as binding IgE in both its secreted and B cell-bound form, CD23 binds CD21 (also known as complement receptor 2), CD18/CD11b (complement receptor 3), CD18/11c (complement receptor 4), and $\alpha_v\beta_3$, the vitronectin receptor. CD23 is a 45 kD type II membrane protein with homology to calcium-dependent (C-type) lectins. It is involved in both the up- and downregulation of IgE synthesis by B cells, augmentation of humoral and cellular responses, and facilitation of the phagocytosis of IgE opsonized antigens. Upon antigen-mediated cross-linking of bound IgE, the low-affinity receptor on B cells downregulates IgE synthesis. Augmentation of IgE-mediated responses can be demonstrated in vivo by the prevention of an immunogen and antigen-specific IgE-induced increase in serum IgE titers following pretreatment with anti-CD23 antibodies. As well as its effects on the FcεRI receptor, IgE can also upregulate CD23 resulting in an increased allergic response in the bronchial mucosa. This is thought to occur via enhancement of allergen uptake and

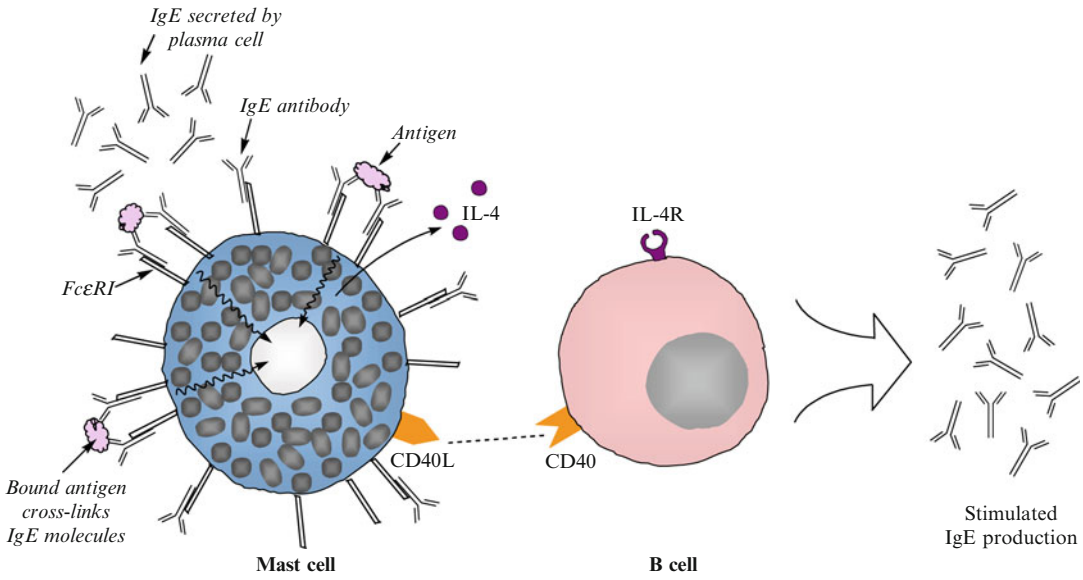


Fig. 3.5 Amplification of IgE antibody production by B cells by direct interaction of mast cells expressing CD40L and secreting IL-4. These interact with their complementary receptors on the B cell surface inducing class switching and the production of IgE antibodies

presentation. Considering the effects of IgE on the high and low IgE receptors, inhibition of the antibody leads to downregulation of both receptors and ultimately decreased mediator release from mast cells and basophils.

Important findings on CD23 control of IgE antibody synthesis and homeostasis in human B cells have recently been forthcoming. The endogenous metalloprotease, a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), demonstrated the existence of two forms of CD23 by releasing its soluble form (sCD23) from membrane CD23 (mCD23). Upregulation of mCD23 in tonsil B cells following treatment with Il-4 and anti-CD40 led to accumulation of sCD23 in the medium prior to class switching to IgE synthesis. Inhibition of mCD23 cleavage by an inhibitor of ADAM10 or small interfering RNA inhibition of CD23 synthesis suppressed IL-4- and anti-CD40-induced IgE synthesis, but addition of recombinant sCD23 enhanced IgE synthesis. Since this occurred even when mCD23 is protected from cleavage, it seems that IgE synthesis is positively controlled by sCD23, and further, sCD23 binds to cells co-expressing IgE and membrane CD21. These results have been interpreted as membrane-bound IgE and CD21 having a role in the sCD23-mediated positive regulation of IgE synthesis with feedback occurring when the concentration of IgE becomes great enough to allow binding to mCD23, thus preventing further release of its soluble form.

3.2.5 Important Mediators of the Type I Immediate Allergic Response

3.2.5.1 Histamine

The reader is also referred to Sect. 4.5.2 for a consideration of the place of histamine in the diagnosis of drug allergies and to Sect. 8.4.1 for a summary of histamine receptors and their relevance to opioid analgesics.

Histamine (2-(imidazol-4-yl)ethylamine) is one of the most intensely studied molecules in all biological systems. This fact, and its appar-

ent myriad physiological and pathological effects, is behind a seemingly ever-expanding literature on an extraordinarily broad spread of activities including its role in inflammatory and allergic reactions; many aspects of the immune response; differentiation; cell proliferation; hematopoiesis; neurotransmission; regulation of circulatory functions, vasodilation, and blood pressure; wound healing; gastrointestinal function; and, no doubt, numerous others yet to be elucidated. In peripheral tissues, more than 90 % of body stores of histamine are found in mast cells and basophils, although there are two other main sources in humans—enterochromaffin-like cells of the gut and histaminergic nerves in the brain. In mast cells and basophils, histamine is stored in granules in association with different anionic proteoglycans—heparin in mast cells and condroitin-4-sulfate in basophils. Upon degranulation elicited by specific IgE antibodies, cytokines, or histamine releasers like compound 48/80, calcium ionophore, *N*-formyl-met-leu-phe, phorbol 12-myristate 13-acetate, and some drugs such as opioid analgesics and neuromuscular blockers, histamine is released from the granules in large amounts with the associated proteoglycan.

3.2.5.1.1 Histamine Biosynthesis and Metabolism

Histamine is synthesized from L-histidine exclusively by the inducible enzyme L-histidine decarboxylase located in the cytosol and widely expressed in the body in various cells including mast cells, basophils, parietal cells, gastric mucosa, neurons, and cells of the central nervous system. The mammalian enzyme requires pyridoxine-5-phosphate as an active site cofactor (Fig. 3.6). Once synthesized, histamine is transported from the cytosol to the secretory granules by vesicular monoamine transporter 2 (VMAT2). L-Histidine decarboxylase is detectable only in cells producing histamine since it is synthesized only when the mediator is required and degraded as soon as synthesis is terminated. Given histamine's pronounced physiological actions, its inactivation to metabolites that do not interact with histamine receptors is a requirement. This is achieved by methylation and oxidation. In mammals, histamine is inactivated in two main ways—methylation of the imidazole ring effected by histamine *N*-methyltransferase (HMT) and oxidative deamination of the primary amino group catalyzed by diamine oxidase (DAO)—to form *N*-methylhistamine and imidazole-4-acetaldehyde, respectively (Fig. 3.6). HMT, which is specific for histamine, is present in most tissues and responsible for the inactivation of intracellular histamine. The enzyme catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to the secondary amino group of the imidazole ring. DAO is stored in secretory vesicles and expressed mainly in intestinal and kidney epithelial cells. Its release is stimulated by heparin which is liberated together with histamine by activated mast cells. Heparin terminates the action of histamine by inactivating it locally. DOA is also active in the gut where it catabolizes histamine present in some foods, thus preventing it from entering the circulation. The products of histamine inactivation by the two different routes are further metabolized (Fig. 3.6). *N*-methylhistamine is converted to *N*-methylimidazole-4-acetaldehyde by mitochondrial monoamine oxidases and this aldehyde, in turn, is catalyzed by aldehyde dehyrogenases to *N*-methylimidazole-4-acetic acid. In the DAO pathway, the first product from the breakdown of histamine, imidazole-4-acetaldehyde, is also catalyzed to the acetic acid derivative by aldehyde dehydogenase before its subsequent ribosylation for transport and excretion.

3.2.5.1.2 Histamine Receptors

The physiological and pharmacological effects of histamine are mediated through four different receptors H_1, H_2, H_3, and H_4, all members of the 7-transmembrane g protein-coupled receptor (GPCR) family with amino terminal glycosylation sites and phosphorylation sites for protein kinases A and C. The receptors are widely expressed on different tissues that are responsive to histamine. For the H_1 receptor these tissues include smooth muscle cells of the airways and vasculature, the gastrointestinal tract, cardiovascular system, neutrophils, endothelial cells, T and B cells, hepatocytes, nerve

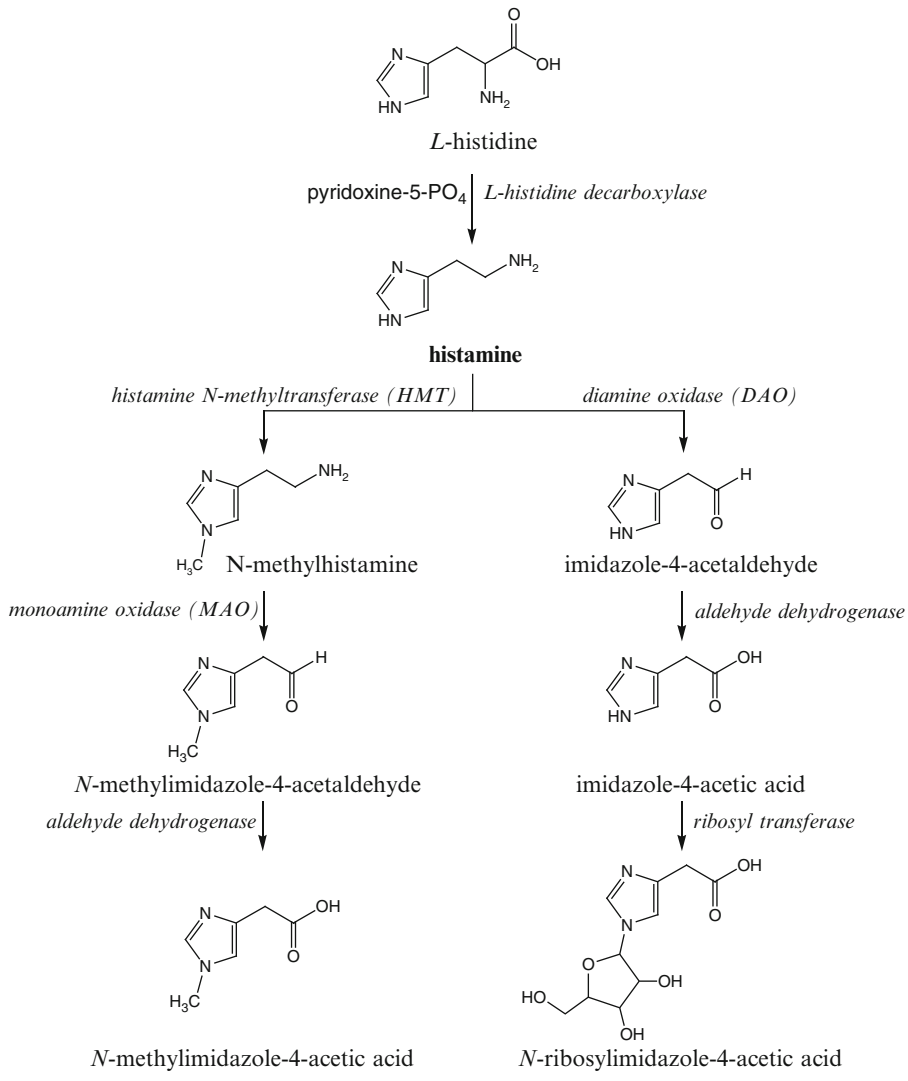


Fig. 3.6 Biosynthesis of histamine from L-histidine by the widely expressed enzyme L-histidine decarboxylase and its metabolism by methylation (via histamine *N*-methyltransferase) and oxidation (via diamine oxidase)

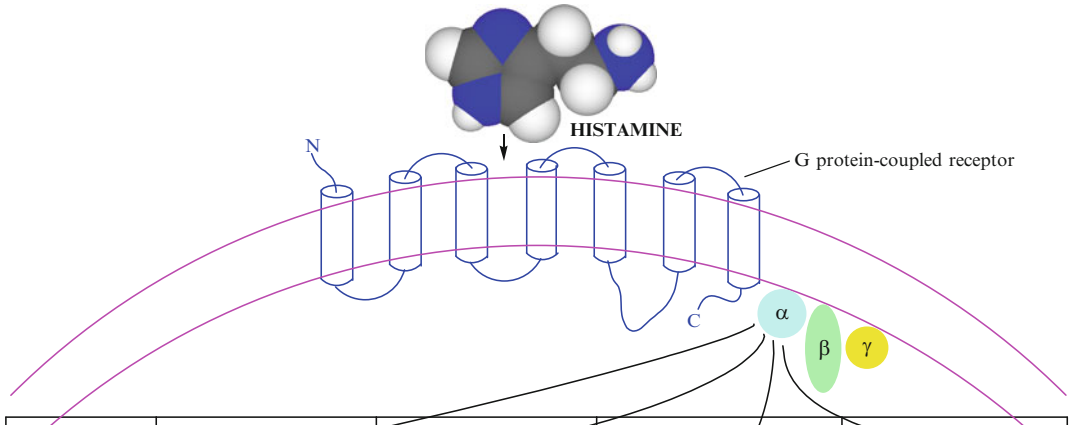
cells, and cells of the genitourinary system suggesting an important role for the autacoid in the modulation of immune, inflammatory, and allergic processes. The H_2 receptor is expressed in gastric parietal cells, the central nervous system, vascular smooth muscle, heart, neutrophils, and uterus. H_3 receptors appear to be less widely distributed occurring in the central and peripheral nervous systems while H_4 receptors are largely expressed in hemopoietic cells where they modulate eosinophil migration and selective recruitment of mast cells. For signal transduction, the H_1 and H_2 receptors activate G_q and G_s-coupled proteins respectively while both H_3 and H_4 are coupled to, and activate, $G_{i/o}$ proteins.

Pathophysiological effects resulting from stimulation of the **H_1 receptor** include those responses seen in immediate allergic reactions, viz, redness, itch, swelling, asthma, anaphylaxis, bronchoconstriction, and vascular permeability. The primary activation of the H_1 receptor, a $G\alpha_{q/11}$-coupled protein, proceeds through phospholipase C which catalyzes the formation of inositol-1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG) from phosphatidylinositol 4,5-biphosphate. IP_3, released into the cytosol, binds to its receptor in the endoplastic reticulum causing an increase in cytosolic Ca^{2+} levels. DAG, acting as a second messenger, activates protein kinase C (PKC). This pathway is activated and proceeds in the brain, airways, and intestinal and vascular smooth muscle. H_1 receptor activation in some other tissues can stimulate adenyl cyclase and cAMP formation. The signaling pathways are not yet fully understood, particularly details of the involvement of Ca^{2+}. Some of the resultant responses in vascular endothelial cells after stimulation of the H_1 receptor and elevated intracellular Ca^{2+} levels are permeability changes, synthesis of prostacyclin and platelet-activating factor (PAF), and release of Von Willebrand factor and nitric oxide (NO).

Whereas H_1 receptors are involved with positive effects, **H_2 receptors** appear to mainly mediate suppressive activities of histamine including gastric acid secretion, heart contraction, cell proliferation, differentiation, and some effects on the immune response. H_2 receptors are coupled to the adenylate cyclase as well as the phosphoinositide second messenger systems via separate GTP-dependent mechanisms, but H_2-dependent effects, particularly those of the central nervous system, are predominantly mediated through cAMP. It has been shown that receptor binding stimulates activation of c-Fos, c-Jun, PKC, and P70S6 kinase. Alternative signaling pathways have been reported (Fig. 3.7). These include a receptor-mediated increase in intracellular Ca^{2+} and/or IP_3 levels in HL-60 human promyelocytic leukemia cells and an increase in cAMP and inhibition of release of arachidonic acid in Chinese hamster ovary (CHO) cells transfected with rat cDNA and induced by calcium ionophore.

The **H_3 receptor** regulates the synthesis and release of histamine and also has a regulatory role in the release of neurotransmitters such as serotonin, dopamine, and norepinephrine. The receptor is expressed in those regions of the central nervous system associated with cognition, in particular, the hippocampus, basal ganglia, and cortical areas, and in the peripheral nervous system, namely, the cardiovascular system, gastrointestinal tract, and airways. The H_3 receptor signals through $G_{i/o}$ proteins and alternative signaling pathways appear to be activated by these proteins. Stimulation of the receptor results in adenyl cyclase inhibition and lower levels of cAMP and PKA. Alternative signaling pathways may be activated including activation of phospholipase A_2 (PLA_2), stimulation of mitogen-activated protein kinase (MAPK), the inhibition of Na^+/H^+ exchange, and K^+-induced Ca^{2+} mobilization. A study of H_3 receptor-mediated attenuation of norepinephrine exocytosis in cardiac sympathetic nerves identified a novel pathway in which stimulation of the receptors on nerve endings produces intraneuronal activation of the MAPK cascade. PLA_2, phosphorylated by MAPK, translocates to the cell membrane where it acts on membrane phospholipids producing arachidonic acid, the substrate for cyclooxygenase and the production of prostaglandin E_2 (PGE_2). PGE_2 activates prostaglandin E receptor 3 (EP_3R) on the cell membrane where the $G\beta\gamma_i$ subunit of EP_3R inhibits Ca^{2+} entry resulting in attenuation of norepinephrine exocytosis. It is apparent that with the H_3 receptor, different signaling can be employed in different cell systems. A further illustration of this is the demonstration of H_3 receptor-mediated activation in the inhibition of the growth of cholangiocarcinoma in vitro and in vivo. Activation of H_3 receptors by a high-affinity H_3 agonist decreased cholangiocarcinoma growth by increasing levels of IP_3, translocation of PKCα, and IP_3/Ca^{2+}-dependent dephosphorylation of the extracellular signal-regulated kinases ERK 1/2.

A new signaling pathway of the H_3 receptor involving receptor modulation of the activity of the serine/threonine-specific protein kinase Akt (protein kinase B, PKB)/GSK-3β (glycogen



RECEPTORS	H_1 GTP— α_q	H_2 GTP— α_s	H_3 GTP— $\alpha_{i/o}$	H_4 GTP— $\alpha_{i/o}$
Main signaling pathway	PLC-β → IP_3 + DAG Ca^{2+} PKC	AC ↑ → cAMP ↑ PKA	AC ↓ → cAMP ↓ PKA ↓	AC ↓ → cAMP ↓ PKA ↓
Alternative pathways	AC ↑ → cAMP ↑	Intracellular Ca^{2+} ↑ & IP_3 ↑ in HL-60 cells cAMP ↑ & AA ↓ in CHO cells	MAPK → PLA_2 → AA → PGE_2 → EP_3R → Ca^{2+} ↓ in guinea-pig cardiac synaptosomes & heart High affinity agonist → IP_3 → PKC_α, IP_3/Ca^{2+}-dephosphylation of ERK1/2 in human cholangiocarcinoma Modulation of Akt/GSK-3β axis in human neuroblastoma	PLC → IP_3 → DAG Ca^{2+} in mouse mast cells Agonist-biased signaling: recruitment of β-arrestin independent of G protein activation in human osteosarcoma cell line U20S

Fig. 3.7 Summarized comparisons of G protein coupling and main and alternative signaling pathways for the four histamine receptors H_1, H_2, H_3, and H_4 (see also Table 3.1)

synthase kinase 3β) axis was recently demonstrated in SK-N-MC cells from a neuroepithelioma cell line. Receptor stimulation with an H_3 agonist induced the phosphorylation of Ser473 and Thr308 on Akt, a kinase important for neuronal development and function. Studies suggested that the Akt activation occurs via a $G_{i/o}$-mediated activation of PI3K (see Sect. 3.2.6.1). H_3 receptor activation also resulted in phosphorylation of Ser 9 on GSK-3β, a ser/thr kinase which acts downstream of Akt. This kinase is important in brain function and this newly identified signaling pathway adds important knowledge to our understanding of the role of H_3 receptor-controlled histamine in brain function. The three above-outlined alternative pathways are summarized in Fig. 3.7.

Following the realization that not all of the biological effects of histamine could be attributed to histamine receptors H_1, H_2, and H_3, a fourth receptor was postulated and **histamine receptor H_4** was subsequently cloned in 2000–2001. Receptor H_4 shows a 35 % amino acid sequence homology with the H_3 receptor and the two are similar in gene structure. The receptor, essentially confined to hemopoietic cells, exerts a chemotactic effect on several cell types associated with immune and inflammatory responses such as allergy, asthma, rheumatoid arthritis, and inflammatory bowel disease and this has led to interest in the development of new agents targeting these diseases. H_4 receptors are functionally expressed on mast cells, eosinophils, monocytes, dendritic cells, and CD8+ T cells. Although the presence

Table 3.1 Summarized comparison of function, G protein coupling and signaling pathways of histamine H_1, H_2, H_3, and H_4 receptors[a]

Receptor	H_1	H_2	H_3	H_4
Best characterized function	Acute allergic reaction	Gastric acid secretion	Modulation of neurotransmitters	Immuno-modulation
Indications for antagonists	Allergy, pruritus[b]	Gastroesophageal reflux disease, peptic ulcer[b]	Sleep and cognition disorders[c]	Pruritus, asthma[c]
G protein coupling	$G_{\alpha q/11}$	$G_{\alpha s}$	$G_{\alpha i/o}$	$G_{\alpha i/o}$
Main signaling pathway	Ca^{2+} ↑	cAMP ↑	Inhibition of cAMP	Ca^{2+} ↑

[a]See also Fig. 3.7
[b]Approved indications
[c]Potential indications

of large amounts of histamine in mast cells and the cell's histamine-releasing properties are well known, expression of histamine receptors on mast cells had not been convincingly demonstrated and there has been little information on the effect of histamine on the cell. It is now known that mast cells express the H_4 but not the H_3 receptor, but exposure to histamine, or histamine in combination with antigen–IgE antibody complexes, does not lead to degranulation of mast cells. The H_4 receptor has, however, been clearly implicated in inflammation and pruritus in animal models. In a rat model of carrageenan-induced acute inflammation, antagonists of the receptor inhibited edema formation and reversed the thermal hyperalgesia. In a histamine-induced itch model in mice, H_4 antagonists inhibited but did not abolish scratching and itch was reduced in H_4-deficient mice. Centrally acting H_1 receptor antagonists produced a partial reduction and combined treatment with both antagonists completely eliminated itch. Further evidence for the involvement of both H_4 and H_1 receptors in histamine-induced itch was the production of itch following administration of agonists of both receptors. There are many mediators of itch and mechanisms are complex. With the belief that the mechanisms underlying itch in chronic conditions such as atopic dermatitis are more likely those associated with mast cell degranulation, a mouse model of itch was set up by injecting antigen-specific IgE intradermally and challenging with antigen 24 h later. H_4 receptor antagonists significantly reduced itch and this was also the result seen in mice deficient in the H_4 receptor. Interestingly, expression of the H_4 receptor on mast cells or any other cell was not required for the pruritic activity, leading to the speculation that H_4 receptor-mediated pruritus may result from actions on peripheral neurons. While the relevance to pruritus in humans of the results with animal models is uncertain, there is optimism that antihistamines specifically targeting the H_4 receptor may lead to more effective treatment of pruritic conditions in humans.

The H_4 receptor is mainly coupled to $G_{i/o}$ proteins and, in common with the H_3 receptor, this leads to inhibition of adenyl cyclase and decreased production of cAMP and downstream effects on cAMP response element-binding (CREB) gene transcription. As with the other histamine receptors, other signaling pathways have been reported (Fig. 3.7). From a study of the signaling pathways of the endogenous mouse H_4 receptor of bone marrow-derived mast cells, histamine activation of the receptor was shown to induce chemotaxis without affecting degranulation of the mast cells. The following interpretations and sequence of events were suggested. Binding of histamine to the receptor on mast cells and eosinophils activates the pertussis toxin-sensitive $G\alpha_{i/o}$ proteins triggering PLC possibly via the G protein $\beta\gamma$ subunits dissociated from the $G\alpha_{i/o}$ proteins. PLC hydrolyzes phosphatidylinositol 4,5-biphosphate to IP_3 and DAG. IP_3 diffuses into the cytosol and binds to its receptor on the endoplastic reticulum where it activates a Ca^{2+} channel causing the release of intracellular

Ca^{2+}. The increased Ca^{2+} triggers mast cell chemotaxis toward histamine by pathways yet to be worked out. It has been suggested that this mechanism might be responsible for mast cell accumulation in allergic tissues.

There is mounting evidence that when the same receptor can activate more than one pathway, some agonists can activate one pathway in preference to another. The need to consider more than one downstream signaling pathway in histamine-GPCR studies was again reinforced by a recent investigation of signaling at the H_4 receptor using the selective antagonist for G protein-dependent signaling JNJ7777120 (1-[(chloro-1-*H*-indol-2-yl)carbonyl]-4-methylpiperazine). Downstream signaling measurements of G protein activation and β-arrestin recruitment demonstrated that the antagonist is what has been described as a biased agonist, acting as an agonist in a non-G protein-dependent manner to recruit β-arrestin to the receptor. β-Arrestin is part of the mechanism for regulating the activity of GPCRs. In stabilizing an alternative active conformation of the H_4 receptor that initiates β-arrestin recruitment but not G protein activation, that is, agonist-biased signaling, JNJ7777120 may be exhibiting the capacity to exist in multiple active conformations. This may result in an agonist stabilizing a slightly different state that preferentially couples to one pathway and not another.

Summarized comparisons of functions, indications for antagonists, G protein coupling, and signaling pathways for the four histamine receptors are shown in Table 3.1 and Fig. 3.7.

3.2.5.2 Cysteinyl Leukotrienes

For a discussion of cysteinyl leukotrienes in relation to diagnostic investigations of suspected drug allergies, see Sect. 4.5.3.

Originally isolated after stimulation of lung tissue by histamine and snake venom and named over 70 years ago as "slow reacting substance of anaphylaxis," (SRS-A), leukotrienes are a family of bioactive peptide-conjugated eicosanoid lipids produced by mast cells, basophils, eosinophils, and macrophages. The name "cysteinyl leukotrienes" is derived from the facts that the compounds are synthesized by leukocytes, they contain three conjugated double bonds or alkenes, and four members of the group, LTC_4, LTD_4, LTE_4, and LTF_4, contain the amino acid cysteine. Although the leukotrienes were originally identified by their contractile effect on smooth muscle, they are now recognized as potent inflammatory mediators with a range of other biologic effects. In particular, LTC_4 and LTD_4 are powerful mediators of asthma, airway hypersensitivity, and allergies, inducing bronchoconstriction, increasing vascular permeability, and promoting mucous secretion. Upon inhalation, both mediators are up to 1,000 times as potent as histamine whereas LTE_4 is only 39 times as potent as histamine in reducing maximum expiratory flow at 30 % of vital capacity. LTE_4, the most stable of the three cysteinyl leukotrienes, is present in greatest amount in vivo where it induces bronchial eosinophilia and airway hyperresponsiveness. Unlike LTC_4 and LTD_4, LTE_4 persists longer in serum, urine, and bronchoalveolar lavage fluid of asthmatics. Urinary excretion of LTE_4 is therefore sometimes used as an indicator of asthma. The bronchoconstriction provoked by LTE_4 is strong in patients with aspirin-sensitive asthma but much weaker in other asthmatics, whereas LTD_4 is much more pronounced in asthmatic patients not sensitive to aspirin (see Chap. 9). Another difference between the two mediators in their effects on asthmatics is the recruitment into sputum of basophils, mast cells, and eosoniphils by LTE_4 but not by LTD_4. LTD_4 aids the adhesion and migration of some cancer cells and increases proliferation of mast cells. All three cysteinyl leukotrienes produce an equiactive wheal and flare reaction characteristic of an allergic response when injected intradermally at a concentration of 1 nmol per site.

3.2.5.2.1 Biosynthesis

As part of the response to leukocyte cell activation, cysteinyl leukotrienes are generated de novo from arachidonic acid liberated from cell membrane phospholipid by cytosolic phospholipase A2 (Fig. 3.8). In concert with 5-lipoxygenase-activating protein (FLAP), the enzyme 5-lipoxygenase (5-LO) converts arachidonic

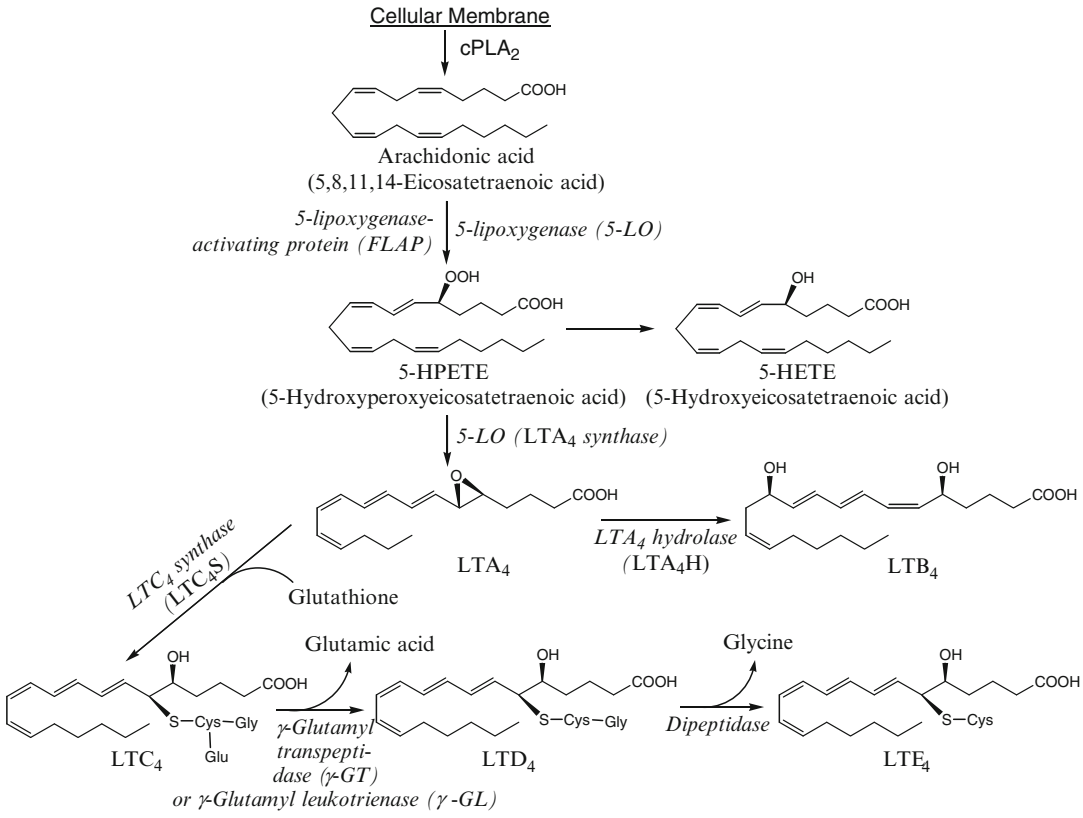


Fig. 3.8 Biosynthesis of cysteinyl leukotrienes from arachidonic acid showing the pathways to the formation of LTA_4, LTB_4, LTC_4, LTD_4, and LTE_4

acid to 5-hydroxyperoxyeicosatetraenoic acid (5-HPETE) which spontaneously reduces to 5-hydroxyeicosatetraenoic acid (5-HETE). 5-LO converts 5-HPETE to leukotriene A_4, an unstable peroxide. Note that the enzyme involved in this step is sometimes referred to as LTA_4 synthase. LTA_4 synthase activity co-purifies with 5-LO and the same cytosolic and membrane-bound active proteins are required for reactions catalyzed by 5-LO and the so-called LTA_4 synthase in crude human leukocyte homogenates leading to the conclusion that a single enzyme is responsible for the production of 5-HPETE from arachidonic acid and for its subsequent conversion to LTA_4. In neutrophils and monocytes which have the enzyme LTA_4 hydrolase, LTA_4 is converted to the dihydroxyacid leukotriene LTB_4, a chemoattractant for neutrophils, whereas in mast cells, basophils, eosinophils, and macrophages, all of which express LTC_4 synthase, LTA_4 is conjugated to reduced tripeptide glutathione to form the cysteinyl leukotriene LTC_4. After transportation to the cell surface in an energy-dependent step with the assistance of multidrug resistance-associated protein 1 (MRP-1), LTC_4 is converted extracellularly to LTD_4 by a γ-glutamyl transpeptidase (γ-GT) or γ-glutamyl leukotrienase (γ-GL). In the final step in the pathway, a dipeptidase removes glycine from LTD_4 producing LTE_4 which is excreted unchanged in the urine. LTF_4 which has an *S*-glutamylcysteinyl group has been prepared in vitro from LTE_4 with glutathione and γ-glutamyltranspeptidase but, as yet, it has not been found in vivo. In comparison to the other cysteinyl leukotrienes, LTF_4 contracts vascular smooth muscle poorly—the rank order of potency being $LTD_4 > LTC_4 > LTE_4 >> LTF_4$. Although leukotriene synthesis generally proceeds

via the 5-LO pathway, a second family of leukotrienes (eoxins, given the prefix EX) can be generated from the action of 15-LO (and 12-LO) first on arachidonic acid and then, for the 15-lipoxygenase compounds, 15-HPETE to form the 15-epoxytriene 15-LTA_4 (EXA_4) followed by the pro-inflammatory cysteinyl 15-leukotrienes 15-LTC_4 (EXC_4), 15-LTD_4 (EXD_4), and 15-LTE_4 (EXE_4) in eosinophils, mast cells, and nasal polyps of allergic subject (see also Sects. 9.4.1, 9.4.3 and Fig. 9.3). IL-4-primed human mast cells incubated with arachidonic acid synthesize and release EXC_4 and possess the capacity to produce EXD_4 cells while nasal polyps spontaneously release EXC_4. Eoxins modulate and enhance vascular permeability, being 100 times more potent in this respect than histamine and almost as potent as LTC_4 and LTD_4. Two types of the 15-LO enzyme are known, 15-LO-1 (which also has about 10 % 12-lipoxygenating activity) and 15-LO-2, both of which produce 15(S)-HETE from arachidonic acid but 15-LO-1 oxygenates arachidonic acid at carbons 15 and 12 while 15-LO-2 adds oxygen only at carbon 15. Human eosinophils and airways epithelial cells contain high amounts of 15-LO-1 as do some subsets of human mast cells, macrophages, and dendritic cells. Expression of 15-LO-2 appears to be restricted to lung, skin, prostate, and cornea.

3.2.5.2.2 Cysteinyl Leukotriene Receptors

Two human cysteinyl leukotriene receptors $CysLT_1R$ and $CysLT_2R$, cloned at the turn of the century, do not bind the three cysteinyl leukotriene ligands equally. The rank order of binding for $CysLT_1R$ is $LTD_4 > LTC_4 = LTE_4$ and for $CysLT_2R$, $LTC_4 = LTD_4 > LTE_4$. The receptors are expressed on a wide range of organ tissues and cell types—$CysLT_1R$ on spleen, lung, small intestine, placenta, bronchial smooth muscle, mast cells, neutrophils, eosinophils, macrophages, monocytes, and hemopoietic progenitor cells and $CysLT_2R$ on lung, heart, lymph node, spleen, brain, bronchial and coronary smooth muscle, adrenal medulla, mast cells, eosinophils, macrophages, and monocytes. The receptors were initially studied with prototypes of the later-to-be-developed "lukast" antagonists. $CysLT_1R$, which bound LTD_4 much more strongly than LTC_4, was competitively blocked by the antagonists while $CysLT_2R$ bound the two cysteinyl leukotrienes with equal affinity and bound LTD_4 at one-tenth the affinity shown by $CysLT_1R$. LTE_4, however, did not display any appreciable binding to either receptor. Despite this, some early recognized pharmacologic properties of LTE_4, viz, its superior potency to its related compounds in contracting guinea pig tracheal smooth muscle and enhancement of this effect produced by histamine, its known peripheral and central airway effects in guinea pigs, and its capacity to increase permeability in guinea pig and human skin, all suggested a distinct pathobiologic role and the existence of a distinct receptor for LTE_4. Studies by K. Frank Austen's group of cysteinyl leukotriene-dependent swelling of ear tissue in mice lacking both CysLT receptors proved the existence of a distinct LTE_4-reactive cutaneous receptor. Ear swelling, a measure of LTE_4-mediated vascular leakage, was inhibited by pretreatment with pertussis toxin or a Rho kinase inhibitor, indicating the involvement of a human GPCR to $G\alpha_i$ proteins and Rho kinase. Until this cutaneous receptor is cloned, it has been designated $CysLT_ER$. Further studies of single receptor-deficient strains of mice compared to wild-type mice showed that the permeability response to LTC_4 or LTD_4 was reduced by half in *Cslt1r*$^{-/-}$ mice but was normal in magnitude and delayed in *Cslt2r*$^{-/-}$ mice. These results suggested that $CysLT_1R$ is the major signaling receptor for LTC_4 and LTD_4 while $CysLT_2$ negatively regulates $CysLT_1R$. Vascular leakage was not reduced by LTE_4 in *Cslt1r*$^{-/-}$ mice but again sustained and delayed in the *Cslt2r*$^{-/-}$ strain, indicating that $CysLT_ER$ is the dominant receptor for LTE_4 and that $CysLT_2R$ once again acts as a negative regulator.

Studies on expression of cysteinyl leukotriene receptors by human mast cells unexpectedly revealed that LTE_4 helps to induce greater numbers of mast cells from cord blood progenitor cells cultured together with IL-6 and IL-10 than

both LTC_4 and LTD_4 and it is also more potent for the production of the inflammatory chemokine macrophage inflammatory protein-1β (MIP-1β) and for the expression of COX-2 and prostaglandin D_2. Sequence homologies of the classical type 1 and 2 cysteinyl leukotriene receptors and the P2Y receptor family together with computer modeling studies indicated that LTE_4 might be a surrogate ligand for a previously unrecognized receptor on mast cells. Human mast cells express the $P2Y_{12}$ receptor, a Gαi-linked receptor for adenosine diphosphate. Subsequent investigations by Austen's group using ovalbumin-sensitized and *Cyslt1r/Clt2r*$^{-/-}$ mice, expression of IL-13, and the $P2Y_{12}$ receptor-selective antagonist clopidogrel suggested that LTE_4 acted as an agonist for platelet activation in the pulmonary vasculature. It seems, therefore, that $P2Y_{12}$ is the receptor for LTE_4-mediated amplification of allergic pulmonary infiltration and proliferation of mast cells and this receptor is separate and distinct from the $CysLT_ER$ in the skin.

3.2.5.3 Platelet-Activating Factor

Platelet-activating factor (PAF), a preformed mediator of anaphylaxis released by degranulating mast cells, is one of the most powerful autacoids yet discovered. The PAF story began in the early 1970s when Benveniste, Henson, and Cochrane demonstrated the release of a substance with both powerful anaphylactic and platelet aggregating properties from allergically sensitized rabbit leukocytes. Although first investigated in relation to anaphylaxis and other allergic manifestations, later studies revealed a wide diversity of other biological actions and involvement in diseases such as asthma, some delayed hypersensitivity reactions, septic shock, adult respiratory distress syndrome, rheumatoid arthritis, necrotic bowel disease, and a wide range of other inflammatory conditions. This diversity of biological actions and pathogenic involvements is due to the mediator's activation of other cells besides platelets, in particular, eosinophils, neutrophils, fibrocytes, neurocytes, and endothelial, vascular, cardiac, smooth muscle, pancreatic, and secretory cells.

3.2.5.3.1 Chemistry and Structure–Activity Relationships

PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a phospholipid of relatively simple but unique structure, belongs to a relatively minor class of lipids, the ether-linked phospholipids. The distinguishing feature of its unique structure is an acetyl group at position 2 of the glycerol backbone (Fig. 3.9). Removal of the acetyl group produces lyso-PAF which is devoid of biological activity. When produced and liberated naturally in the cellular environment, PAF is made up of a mixture of homologs differing in the number of carbons and the degree of unsaturation of the alkyl chain at position 1 of the glycerol backbone. The main homologs usually present are the $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ structures. The structures for maximum activity are a 16 carbon chain, the 1-*O*-alkyl ether linkage, the acetyl group at position 2, the *sn* configuration, and the phosphate group at carbon 3 (Fig. 3.9). Activity decreases progressively as the C chain backbone is shortened or lengthened; replacement of the ether linkage leads to no or little biological activity; the unnatural enantiomer with the *(S)*-configuration is inactive; for any biological activity the only substituents tolerated at carbon 2 are propionyl and *N*-methyl carbamoyl groups (the 2-ethoxy analog has only 10 % of the activity of PAF); and fairly major modifications of the substituents on the nitrogen diminish activity. These specific structural requirements suggest that PAF exerts its biological effects by binding to specific receptors and this is in fact so.

3.2.5.3.2 Biosynthesis and Cellular Sources of PAF

Because PAF is such a potent mediator of a range of biological effects, its concentration in body fluids and tissues needs to be restricted to avoid adverse or even lethal consequences. This is achieved intracellularly and extracellularly by a specific acetylhydrolase and by regulation of the conversion of precursor molecules. The activity of PAF acetylhydrolase for its substrate is extremely high ensuring that the half-life of the mediator in blood is of the order of only a few minutes. PAF is synthesized by two metabolic

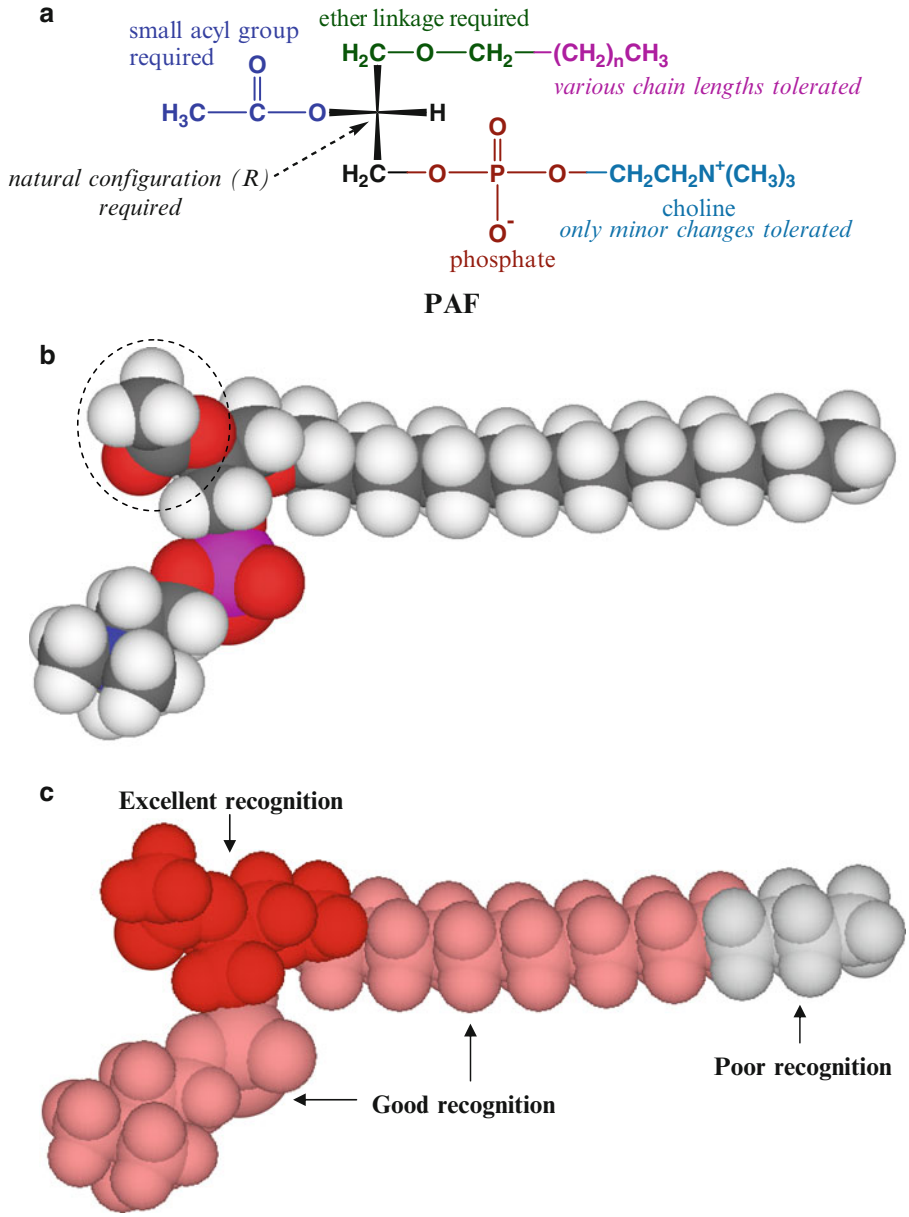


Fig. 3.9 (**a**) Two-dimensional structure of PAF highlighting the important structural features necessary for maximum biological activity and recognition by anti-PAF antibody combing sites. (**b**) Three-dimensional space-filling CPK model of PAF with the acetyl group *circled*. Removal of this group alone produces a molecule devoid of biological activity. (**c**) Outline of PAF model indicating regions of excellent, good, and poor recognition by anti-PAF antibodies. The antibody recognition pattern is very similar to that of the PAF receptor (see Smal MA, Baldo BA and Harle DG. J Mol Recogn 1990; 3: 169–73)

pathways—the de novo and remodeling pathways. In the de novo pathway (Fig. 3.10a), the specific enzyme alkylacetylglycerol cholinephosphotransferase, widely distributed in tissues on the cytoplasmic surface of the endoplasmic reticulum, catalyzes the reaction between 1-*O*-alkyl-2-acetyl-*sn*-glycerols and cytidinediphosphocholine (CDP-choline) in the presence of

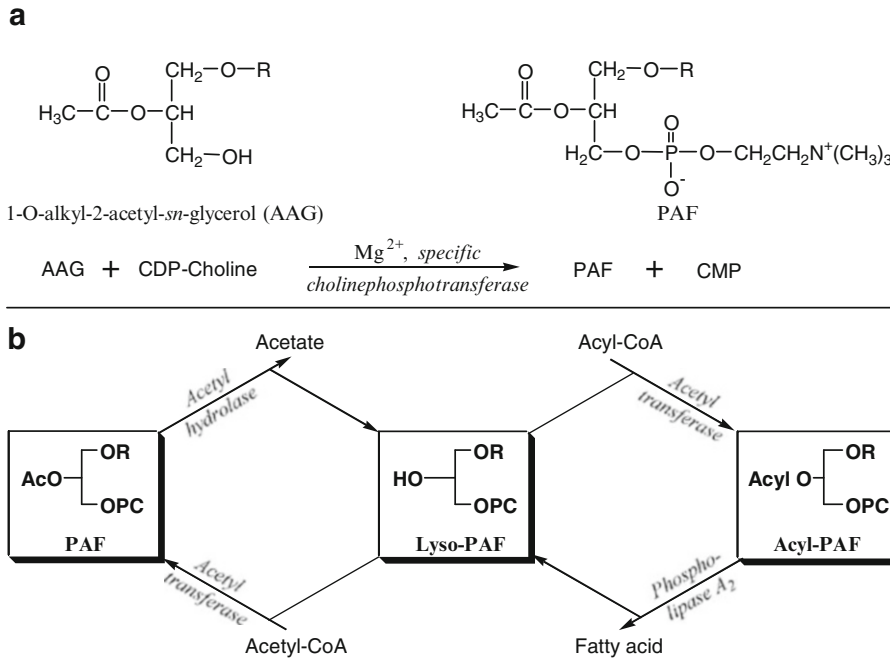


Fig. 3.10 The two biosynthetic pathways for the synthesis of PAF. (**a**) The de novo pathway; (**b**) the remodeling pathway

Mg^{2+} generating PAF and cytidinemonophosphate. This synthetic pathway appears to maintain PAF levels for normal physiological processes. The remodeling pathway (Fig. 3.10b) both activates and deactivates PAF via the calcium-dependent enzymes phospholipase A2 and acetyltransferase, the latter being the rate-limiting enzyme. These enzymes are found particularly in cells of the immune system such as basophils, eosinophils, platelets, polymorphonuclear cells, macrophages, and endothelial cells and can be stimulated by a variety of agents including immune complexes, thrombin and histamine.

3.2.5.3.3 Biological Actions of PAF and Its Role in Health and Disease

PAF is a hydrophobic molecule and for crossing cell membranes and transportation to its various sites of action, serum albumin serves a carrier function. When injected into mammals, PAF produces both the signs and symptoms of anaphylaxis with hypotension, increased vascular permeability and hemoconcentration, thrombocytopenia, neutropenia, and eventually death. Infusion of PAF into the heart decreases myocardial contractility and coronary flow, effects resembling cardiac anaphylaxis. Intradermal injection produces a biphasic inflammatory response similar to the response of allergic subjects to allergen. PAF has a profound effect on the lung producing bronchoconstriction, edema, and hyperresponsiveness. PAF is also one of the most powerful ulcerogenic agents known, provoking hemorrhage and vascular congestion in both the stomach and small intestine.

PAF has been implicated in many disease states but since it is often only one of a range of other mediators present, any preeminent role is understandably often difficult to establish. For example, it is frequently present along with histamine, numerous metabolites of the cyclooxygenase and lipoxygenase pathways, and a range of chemokines and cytokines including TNF. As well as its undoubted role in anaphylaxis and some other allergic reactions, PAF is an important mediator in the asthmatic response.

Administration of PAF into the lungs produces severe bronchoconstriction, mucous secretion, inflammation, and long-lasting airway hyperreactivity. The latter two effects may be contributed to by PAF-induced recruitment and activation of inflammatory cells such as macrophages and eosinophils. Recent findings, particularly in studies in the mouse, have identified a second pathway of anaphylaxis involving the IgG receptor FcγRIII and the release of PAF as the major mediator (see Sect. 3.2.7). Although long suspected, a central role for PAF in anaphylaxis is confirmed and explained by this alternative pathway. In a model of peanut allergy for example, although both histamine and PAF are involved in the response, PAF is more important in shock pathogenesis. Along with anaphylaxis and asthma, septic shock is a disease in which PAF is suspected of having a leading role. PAF induces systemic responses similar to those provoked by bacterial endotoxin and is found in the spleen and peritoneum of rats with endotoxic shock. Some PAF antagonists protect animals against septic shock caused by infection with gram-negative organisms or injection of endotoxin. Because of its potent effect on platelets, PAF is thought to be involved in some thrombotic diseases including stroke. Other suspected roles are in acute graft rejection and immune complex deposition in, for example, systemic lupus erythematosis, psoriasis, and other allergic conditions.

3.2.5.3.4 The PAF Receptor

The PAF receptor is a MW 48 kD, G-protein-coupled single 342 amino acid protein that shows structural characteristics of the rhodopsin gene family. The human, guinea pig, and rat receptors have been cloned and characterized as a seven-transmembrane receptor that induces phosphoinositol turnover. The receptor shows wide tissue distribution being expressed in lung, kidney, liver, spleen, small intestine, and brain. In leukocytes, it is expressed on platelets, neutrophils, monocytes, and B cells but not on resting T cells and natural killer cell lines. Human monocytes treated with IFN-γ show a two- to sixfold increase in PAF receptor expression compared to untreated cells.

3.2.5.3.5 Measurement of PAF in the Laboratory

Accurate measurement of the very small amounts of PAF in fluids and extracts is a prerequisite for studying the role of the mediator in health and disease. The most widely used method relies on the interaction of PAF with platelets, but the procedures are not strictly quantitative, lack specificity, and are difficult to standardize and reproduce; fresh platelet suspensions are required; and throughput capacity is poor. Mass spectrometric techniques are sensitive and specific but the specialized nature of the equipment, absence of easy access for many laboratories, and difficulty of assessing large numbers of samples side by side make this method problematic for routine use by many researchers. Other methods such as measurement of ^{3}H-serotonin after PAF-induced platelet degranulation and radioreceptor assays are specialized procedures in some laboratories but can be difficult to standardize, require cell labeling, or membrane preparations and may show high nonspecific binding. Perhaps the best all-round, high-throughput procedure for quantitating PAF levels in research and test samples is a specific immunoassay, available since the first such assay was introduced in the authors' laboratory in 1989. This method is highly specific, sensitive in the range 10–1,000 pg (0.02–2 pmoles), has a high capacity, and is not affected by inhibitors of platelet aggregation.

3.2.6 Anaphylaxis

Anaphylaxis is a sudden, systemic reaction involving a number of different organs of the body that may be severe enough to cause death. It is usually provoked by exposure to allergens with drugs and foods being the most common causes. For the clinical features of anaphylaxis the reader is referred to Chap. 2. Progress continues in identifying key intermediates and elucidating mechanisms of regulatory systems and signaling pathways during mast cell activation and degranulation and some impressive advances in our understanding of the pathways, the mediators involved, and their contribution to the pathobiology of anaphylaxis are under way.

3.2.6.1 Mechanisms of FcεRI-Mediated Mast Cell Activation in Anaphylaxis

Understanding anaphylaxis involves study of the cellular events leading to the release of mediators of inflammation and hypersensitivity with emphasis on the mechanisms, in particular the signaling processes, of mast cell and basophil activation and degranulation. Upon activation of mast cells and basophils following cross-linking by allergen of receptor-bound IgE and aggregation of the high-affinity IgE FcεRI receptors, the cells quickly release preformed mediators from the secretory granules. These mediators, including histamine, leukotrienes, PGD_2, PAF, and TNF, causing vasodilation, increased vascular permeability and heart rate, bronchoconstriction, airway remodeling, pulmonary and coronary vasoconstriction, and a host of other detrimental effects, including cell recruitment with cytokine and chemokine production, are responsible for the pathophysiology of anaphylaxis. Cross-linking initiates the signaling cascade that ultimately results in anaphylaxis. FcεRI receptor aggregation causes Lyn, the tyrosine kinase associated with the β and γ subunits of the receptor, to phosphorylate the tyrosines of the ITAMs of these two subunits. The phosphorylated ITAMS, mainly on the γ subunit, then act as scaffolds for binding the cytoplasmic tyrosine kinase Syk. As outlined above in Sect. 3.2.2, recruitment of the Syk kinase and subsequent phosphorylation activation steps involving Lyn lead to mast cell activation demonstrating the importance of protein tyrosine kinases in the pathways that result in allergic inflammation and anaphylaxis. These pathways involved in mast cell triggering are summarized in a simplified form in Fig. 3.11. Activated Syk is involved in the phosphorylation of the transmembrane adaptor linker for activation of T cells (LAT) as well as the SH2 domain-containing leukocyte-specific

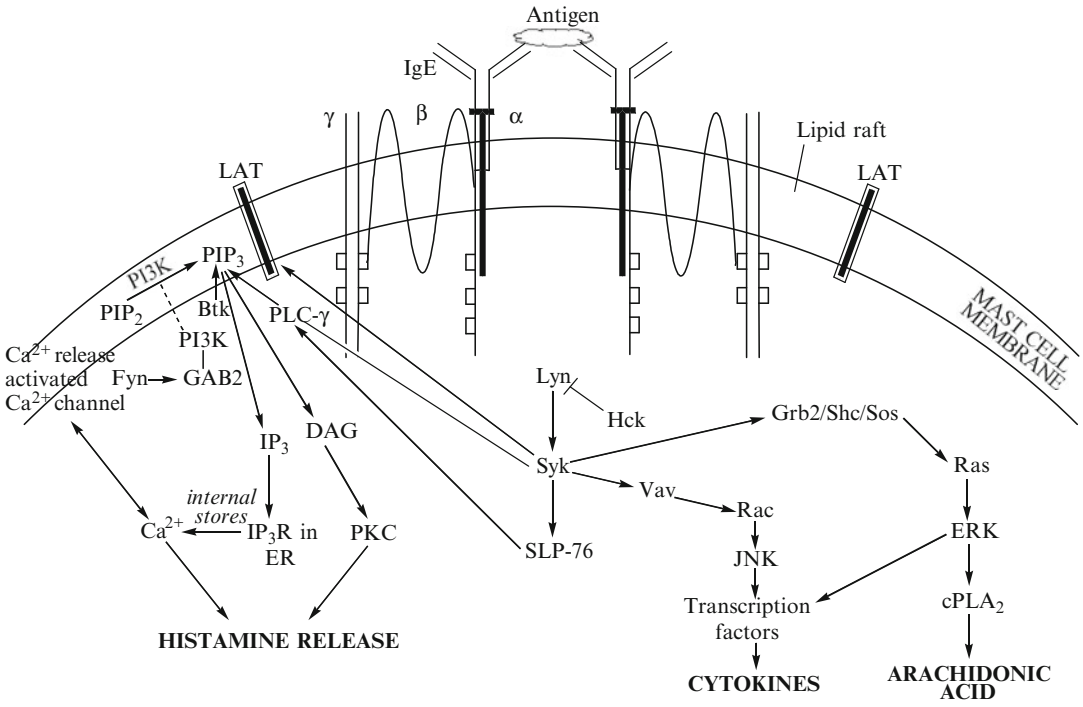


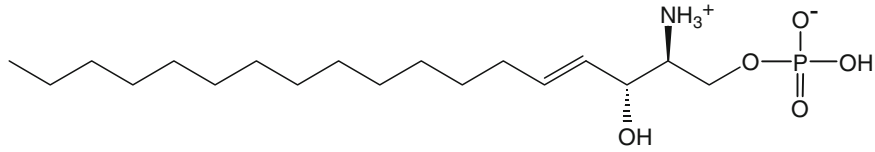
Fig. 3.11 Simplified summary of FcεRI-mediated signaling pathways in the mast cell leading to allergic inflammation and anaphylaxis

protein of MW 76 kD (lymphocyte cytosolic protein 2 LCP2 or SLP-76), the guanine nucleotide exchange factor Vav, phospholipase C-γ1 (PLC-γ1), and PLC-γ2. After involvement of the enzyme proto-oncogene tyrosine-protein kinase (Fyn), tyrosine phosphorylated GAB2 (GRB2 [growth factor receptor-bound protein 2]-associated-binding protein 2) binds a subunit (p85) of phosphatidylinositol 3-kinase (PI3K). In the membrane, PI3K catalyzes the conversion of phosphatidylinositol-4,5-diphosphate (PIP_2) to phosphatidyl-3,4,5-triphosphate (PIP_3). This attracts a number of proteins containing the pleckstrin homology (PH) domain, a 120 amino acid domain occurring in a variety of proteins involved in intracellular signaling. The attracted proteins include Bruton's tyrosine kinase (Btk) and the PLCs γ_1 and γ_2 which in tyrosine-phosphorylated form catalyze the hydrolysis of PIP_2 to inositol-1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG). Both compounds act as second messengers, the former releasing Ca^{2+} resulting in a depletion of Ca^{2+} stores and entry of Ca^{2+} from the extracellular medium and the latter activating protein kinase C (PKC). These events lead to mast cell degranulation. This activity takes place in two regions on the inner side of the plasma membrane. Electron microscopy has revealed a primary region of activity near the FcεRI receptor involving Gab2, the p85 subunit of PI3K, and PLC-γ2 and a second region near LAT involving PLC-γ1 and the p85 subunit. Tyrosine phosphorylation and activation of other enzymes and adaptors, including Vav, Grb2, the SHC-adaptor protein (Shc) involved in signaling, and Son of sevenless (Sos) protein (a guanine nucleotide exchange factor), stimulate the small GTPases Ras, Rho, and Rac. These reactions lead to activation of the extracellular-signal-regulated kinase ERK, Jun amino-terminal kinase JNK, the p38 mitogen-activated protein (MAP) kinase cascade, and histamine release. Phosphorylation of the transcription factors activating protein-1 (AP-1), nuclear factor of activated T cells (NFAT), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) induces the synthesis of cytokines and the activation of cytoplasmic phospholipase A2 (PLA-A2) to release arachidonic acid with the production of lipid mediators (Fig. 3.11).

Further research, much of it in mice, has continued to highlight the key role of tyrosine kinases in FcεRI activation and the subsequent signaling events, although other involved tyrosine kinases such as Hck have been identified. The intensity of stimulation of the FcεRI receptor has been shown to be important. Low-intensity stimulation by IgE with low antigen concentration or by anti-IgE positively regulates mast cell degranulation and the production of cytokines by inhibiting Lyn activity. High-intensity stimulation with high IgE and high antigen concentrations negatively regulates mast cells by enhancing Lyn activity and increased Syk activation. Genetic variation appears to influence the role of tyrosine kinases. For example, an epilepsy- and anaphylaxis-prone strain of mice was found to be deficient in the expression of Lyn while a related epilepsy-prone variant proved anaphylaxis resistant. Bone marrow-derived mast cells (BMMCs) from the anaphylaxis-sensitive mice had reduced Lyn and Syk activities and showed degranulation typical of BMMCs of phenotype Lyn(−/−) whereas the phenotype of the anaphylaxis-resistant mice was similar to wild-type animals.

3.2.6.2 Sphingosine-1-Phosphate, an Emerging Mediator of Anaphylaxis

Activated Fyn, involved in a second tyrosine kinase pathway, has been shown to be required for cytokine production as well as degranulation and to have a role in generating sphingosine-1-phosphate (S1P) (Fig. 3.12) a blood-borne bioactive lipid mediator that is a major regulator of the vascular system and B and T cell trafficking. S1P from mast cells and circulating S1P from macrophages, platelets activated by the release of PAF, endothelial cells, and many other nonimmune cells are elevated in the lungs of asthmatics where they regulate pulmonary epithelium permeability and are thought to contribute to the pathogenesis of asthma and diseases such as rheumatoid arthritis. The detection of elevated S1P levels in bronchial alveolar lavage fluid of challenged asthmatics and demonstration that it is necessary



Sphingosine-1-phosphate

Fig. 3.12 Structure of the bioactive signaling phospholipid sphingosine-1-phosphate, a regulator of immune and vascular systems

for sustained mast cell degranulation through the $S1P_2$ receptor were indications that this lysosphingolipid has a previously unrecognized relationship with anaphylaxis. It is produced by sphingosine kinase (Sphk1 and Sphk2)-catalyzed addition of phosphate to sphingosine, broken down by a S1P lysate and converted back to sphingosine by S1P phosphatase. Recent research has shown that susceptibility to anaphylaxis appears to be due to S1P generated within the mast cell and by free, circulating S1P from non-mast cell sources. The gene SphK2 regulates the influx of Ca^{2+} into mast cells and the responses to it, making it a determinant of intrinsic mast cell function whereas SphK1 appears to act extrinsically affecting mast cell responsiveness by regulating levels of circulating S1P. The surprising demonstration of a relationship between circulating levels of S1P and anaphylaxis is made more intriguing by the demonstration that reduced S1P levels due to a deficiency of SphK1 are associated with resistance to anaphylaxis. While it is well known that only a small number of individuals from a large group with similar circulating levels of allergen-specific IgE antibodies will experience anaphylactic shock when challenged with the allergen, the amount of circulating S1P might help to provide the explanation. Finally, as exciting as these developments in our understanding of the underlying mechanisms of anaphylaxis are, it should be remembered that many of the findings result from research on mice not man and that is also true for most of the S1P studies where mice with SphK1 and 2 genes, individually or jointly deleted, were used. Given the diversity of mast cells and differences in gene expression of mouse and human mast cells, the roles of SphK1 and SphK2 may prove to be significantly different in the two species.

3.2.7 Other Mechanisms of Anaphylaxis: IgG, PAF, and Nitric Oxide

Mechanisms of anaphylaxis independent of IgE have been suggested, for example, anaphylatoxins produced during complement activation, generation of immune complexes, the involvement of T cell activation and cytotoxicity, release of neuropeptides, and a number of different mechanisms acting coincidently without the involvement of allergen-specific IgE. Intriguingly, anaphylaxis can occur in the mouse via the classic pathway involving allergen-induced cross-linking of mast cell FcεRI receptor-bound IgE antibodies with release of histamine (and other mediators) but also by an IgG pathway in which allergen–antibody complexes activate macrophages by cross-linking FcγRIII receptors and with PAF as the main mediator of anaphylaxis. Although there is, as yet, no compelling evidence for an IgG-mediated mechanism in humans, what appears to be anaphylaxis has been described in a few cases where there is an apparent absence of mast cell degranulation, that is, with no increase in serum tryptase. Certainly there are many similarities between the immune systems of mice and men; PAF is produced by macrophages of both species, it has the same affect on vascular permeability, and consequently allergen–IgG complexes may have an important role in anaphylaxis in

humans as well as the mouse. However, human anaphylaxis tends to result from low-dose exposure whereas mouse IgG-mediated anaphylactic reactions may occur in response to relatively larger antigen doses and/or adjuvants that elicit IgG as well as IgE antibodies. What may be evidence in favor of anaphylaxis in humans independent of IgE are the responses seen in patients after receiving the chimeric mouse-human anti-TNF monoclonal antibody infliximab (see also Sect. 11.1.3.3). None of the subjects appeared to have complementary IgE antibodies, all had IgG to the mouse immunoglobulin determinants, and there was no increase (at only 20 min) in serum tryptase. From insights gained from his extensive studies of mechanisms of anaphylaxis in mouse models, F. D. Finkelman has suggested that large doses of antigen might be used in humans to look for evidence of anaphylaxis accompanied by macrophage activation and PAF secretion.

PAF contributes to hypotension and cardiac dysfunction during shock and stimulates, via its receptor, a number of signaling pathways including those that activate PLA_2 and PI3K. Studies of PAF and anaphylactic shock in mice have shown that PAF-induced shock depends on PI3K signaling and on NO produced by constitutive nitric oxide synthase (eNOS) not the inducible form of the enzyme (iNOS). Mouse models showed that inhibition of NOS, PI3K, or Akt, or deficiency of eNOS, gave complete protection against anaphylaxis. These findings appear to support the belief that eNOS has a detrimental role in vascular function during shock and in regulating inflammation. Further, if eNOS-derived NO is the principal vasodilator in anaphylactic shock, eNOS and/or PI3K or Akt might prove to be important targets for treating anaphylaxis.

Clearly, there is much to learn and understand about anaphylaxis and the list of interesting questions that remain unanswered is disconcertingly extensive. The following topics are suggested as important and potentially productive areas of investigations that could be near the top of any current research agenda for anaphylaxis:

- Relationships, if any, between the risk of anaphylaxis and levels of allergen-specific IgE and the affinities of IgE antibodies.
- The relationship between mediator activity and their turnover, for example, PAF acetylhydrolase may be less active in some individuals allowing PAF to remain active for longer.
- Further studies of the relationship of sphingosine-1-phosphate with anaphylaxis.
- Continuing searches for more mediators and markers of anaphylaxis, especially more sensitive ones.
- Is there an IgG-dependent pathway for anaphylaxis in humans? If so, what is the mechanism?
- Identification of signaling pathways that stimulate or inhibit anaphylaxis and how these pathways can be manipulated.
- Further studies on the roles and importance of NO, eNOS, and iNOS.
- The role of the heart in anaphylaxis, in particular, the heart mast cells in cardiovascular collapse.
- Estimation of levels and searches for polymorphisms of relevant cytokines and cytokine receptors such as IL-4, IL-13, and TNF.
- The role, if any, of IgG blocking antibodies.

This list is far from exhaustive.

3.2.8 Drug-Induced Urticaria and Angioedema

For the clinical manifestations of urticaria, see Sect. 2.2.1.2.

Of the drugs implicated in provoking urticaria and angioedema, the NSAIDs are perhaps the most important. What is currently understood of their proposed mechanisms of action together with a review of the arachidonic acid cascade is considered in Chap. 9. Formation of the cysteinyl leukotrienes is detailed in Sect. 3.2.5.2 (above) and is also referred to in Chap. 9.

Urticaria may be classified as acute or chronic. The acute form appears early after exposure, perhaps within minutes, and can last from hours

to several weeks whereas the chronic form persists for about six weeks or more. Urticaria is often an isolated event but drug-induced urticaria, regarded as one of the most common cutaneous drug reactions, can be seen in association with anaphylaxis, angioedema, and serum sickness. Urticaria is a heterogeneous disease with many subtypes caused by a range of agents and stimuli. Some infections (e.g., *Helicobacter pylori*), intolerance to foods, and autoantibodies to the high-affinity IgE receptor FcεRI have been implicated, but, apart from the NSAIDs and angiotensin-converting enzyme (ACE) inhibitors, there is a dearth of information on mechanisms underlying drug-induced urticaria and angioedema.

3.2.8.1 Genetic Mechanisms

Knowledge of drug-induced mechanisms of urticaria and angioedema is limited and this is even more apparent when considering current progress on the molecular genetic mechanisms involved. Information on genetic polymorphisms of relevant genes together with supporting functional studies is needed to help elucidate molecular mechanisms and identify genetic markers. Some progress made in identifying HLA alleles and promoter polymorphism genetic markers for aspirin-induced urticaria/angioedema is covered in Chap. 9, Sect. 9.5.5.

3.2.8.2 Urticaria Due to Immune Mechanisms

Urticaria following drug administration may occur without previous exposure to the drug or after previously tolerated exposures. Drugs appear to cause only a minority of cases of chronic urticaria and while they are often assumed to be the cause of a high proportion of cases of acute urticaria during drug treatment, some results cast doubt on this. An examination, including skin testing, of 350 patients with suspected drug-induced reactions made up of 343 with urticaria/angioedema and seven with anaphylaxis revealed that only 22 (6.3 %) were allergic and had a positive skin test to the suspected drug. The positive reactors proved to be the patients who presented with the most severe symptoms. An immediate (within 20 min) positive skin test is usually presumed to result from an IgE antibody-mediated mechanism or a direct histamine-releasing effect but one cannot necessarily presume that these are the only mechanisms operative in all cases of drug-induced acute urticaria. Symptoms of urticaria are caused by the mediators histamine, leukotrienes, prostaglandin D2, bradykinin, and other vasoactive substances released from mast cells and basophils into the skin. Cases of acute urticaria may be immune or nonimmune mediated. Drug-induced immune-mediated reactions can be elicited by cross-linking of high-affinity (FcεRI)-bound IgE antibodies on mast cells and basophils by free drug or drug–carrier complex molecules reacting with the bivalent antibody combining sites via their complementary allergenic determinants. This results in degranulation of the cells and histamine release.

Other hypersensitivity responses may lead to urticarial reactions. A rare cause of the acute form is a type II hypersensitivity cytotoxic reaction mediated by cytotoxic antibodies and complement activation. An example of this type of reaction occurs in transfusion reactions when IgG and IgM antibodies activate complement and lyse transfused incompatible red cells. Urticaria may also result from a type III antigen–antibody complex-mediated hypersensitivity reaction, in particular, serum sickness lasting for several weeks and presenting with fever, arthralgias, and glomerulonephritis as well as urticaria. Note that there is a drug-induced serum sickness-like reaction that is not associated with circulating immune complexes. Drugs implicated in these reactions include penicillins, cephalosporins, tetracyclines, quinolones, sulfonamides, NSAIDs, carbamazepine, thiouracil, allopurinol, and barbiturates. Other drug-induced type III hypersensitivity reactions involving skin inflammation include erythema nodosum leprosum induced by dapsone and the Jarisch–Herxheimer reaction following treatment of some microorganisms (e.g., in syphilis) with antimicrobials such as penicillins and tetracyclines. The inflammatory cytokines TNF, IL-6, and IL-8 appear to be released in these reactions. Urticarial vasculitis is

another type III hypersensitivity skin eruption that can resemble urticaria and which is sometimes drug induced. Antigen–antibody complexes formed in the vascular lumina lead to complement activation, chemotaxis of neutrophils, and the release of proteolytic enzymes that damage the vascular lumina. Drugs implicated include ACE inhibitors, penicillins, sulfonamides, thiazides, and the antidepressant fluoxetine. Urticarial reactions are also sometimes seen along with other skin manifestations during some drug-induced type IV hypersensitivity responses, but the presence of other skin manifestations, frequently more severe, makes it difficult to distinguish and study the specific mechanisms.

3.2.8.3 Urticaria with an Autoimmune Basis

A significant proportion of cases of chronic urticaria demonstrate no connection with drugs. Observations during the 1980s of the association of chronic urticaria and angioedema with thyroid autoimmunity and on the prevalence of anti-IgE autoantibodies in urticarial syndromes suggested that autoimmunity might have a role in some cases of chronic urticaria. These findings led to the occasional demonstration of the presence of IgG anti-immunogloulin E autoantibodies and functional autoantibodies against the alpha subunit of the high-affinity IgE receptor (i.e., FcεRIα) in at least one-third of patients with chronic urticaria. These autoantibodies activate normal cell function by cross-linking the receptors on cutaneous mast cells and blood basophils, thus releasing histamine and other mediators responsible for the urticaria and angioedema. Activity of the autoantibodies was later shown to be augmented by complement activation with a critical role for component C5a. Chronic urticaria is now divided into autoimmune and idiopathic subgroups since in about 55–60 % of patients the etiology remains obscure. As well as releasing histamine and leukotrienes from basophils, sera with the autoimmune antibodies also release IL-4. A study of lymphocytes from patients with chronic urticaria showed that activated CD4+ T cells produced high amounts of IL-4 and IFN-γ, strengthening the evidence for an immune basis of the disease and supporting histological demonstrations of predominant CD4+ T cell infiltrates in biopsies of chronic urticaria lesions. The observed cytokine profile of Il-4, IL-5, and IFN-γ does not reflect a predominance of Th1 or Th2 cells and cellular infiltrates indicate a Th0 profile or a mixture of activated Th1 and Th2 cells.

Omalizumab, a recombinant humanized monoclonal antibody that inhibits the binding of human IgE to its high-affinity receptor FcεRI by selectively binding the immunoglobulin in solution, has been used as a successful treatment of intractable allergic asthma. The efficacy of the monoclonal antibody treatment was therefore investigated in patients with chronic autoimmune urticaria who remained symptomatic on antihistamine therapy. Of the 12 patients treated, seven showed complete resolution of the urticaria, four responded with a decrease in the urticaria activity score but the urticaria persisted, and one patient showed no improvement.

3.2.8.4 Basophils in Chronic Urticaria

CD203c is a basophil activation marker that is upregulated by cross-linking the FcεRI receptors on mast cells and basophils. Incubation of basophils with sera from patients with chronic idiopathic urticaria and a positive autologous serum skin test (ASST; an intradermal test with the patient's own serum) demonstrated significant upregulation of CD203c and this upregulation correlated with basophil histamine release and the ASST. Basophils from chronic urticaria patients are less responsive to anti-IgE and C5a but highly responsive when incubated with sera, even normal sera. The stimulatory factor(s) in serum has not been identified and the increased response of the cells is not yet understood. In a flow cytometric evaluation of the expression of basophil cell surface markers CD203c, CD63, CD123, and the receptor FcεRIα, both CD203c and CD63 were upregulated on basophils from patients with chronic idiopathic urticaria regardless of their ASST response. High expression of IL-3 receptor on basophils and activated T cells was detected only in ASST-positive patients.

3.2.8.5 Nonimmune-Mediated Urticaria and Angioedema

Angioedema does not always have an allergic basis. Nonallergic angioedema not involving IgE antibodies and unassociated with urticaria can occur. The prototype example is hereditary angioedema arising from a deficiency of the inhibitor for C1 esterase that results in the maintenance of undegraded bradykinin. Acquired angioedema may be due to the accelerated consumption of C1 esterase inhibitor or, with an immune component, to autoantibody production. During acute attacks of hereditary and acquired angioedemas, plasma bradykinin has been shown to rise to up to 12 times the normal level.

3.2.8.5.1 Drugs that Directly Trigger Mast Cell Release

These reactions are sometimes referred to as pseudoallergic responses since their clinical course and presentation are similar to allergic urticaria and angioedema. Drugs including the antimicrobial vancomycin (Chap. 6), neuromuscular blockers used in anesthesia (Chap. 7), opioid analgesics such as morphine (Chap. 8), NSAIDs (Chap. 9), radiocontrast media (Chap. 10), and a wide range of other less often used medications can trigger urticaria by directly stimulating mast cell degranulation and histamine release. The mechanism of mediator release by the NSAIDs is particularly interesting. The drugs inhibit cyclooxygenase which in turn leads to overproduction of the vasoactive and pro-inflammatory leukotrienes (Sect. 3.2.5.2 above). This subject is discussed in more detail in Chap. 9.

3.2.8.5.2 Angiotensin-Converting Enzyme (ACE) Inhibitors and Angioedema

ACE occurs as somatic and germinal isozymes. The somatic enzyme, expressed in the lungs and in vascular endothelial, kidney, and testicular Leydig cells, is part of the renin–angiotensin–aldosterone system, one of the body's mechanisms for maintaining blood pressure. ACE has two actions—it catalyzes the conversion of the ten amino acid peptide angiotensin I to the potent vasoconstrictor eight amino acid peptide angiotensin II and degrades bradykinin, a potent vasodilator. The vasoconstrictor action of angiotensin II may lead to increased blood pressure and hypertension, the effect that led to the development and application of the ACE inhibitor drugs. ACE inhibitors increase bradykinin levels and prolong its action and decrease angiotensin II levels (and therefore a decrease in aldosterone secretion from the adrenal cortex) leading to dilation of blood vessels and a coincident decrease in arterial blood pressure. ACE inhibitors, now widely used to treat hypertension, congestive heart failure and diabetic nephropathy include the drugs benazapril, captopril, enalapril, fosinopril, lisinopril, perindopril, quinapril, ramipril, and trandolopril. The combination of actions of decreasing angiotensin II and aldosterone levels and increasing and maintaining bradykinin levels may lead to fluid extravasation into subcutaneous tissue ultimately producing angioedema. The increased levels of bradykinin are thought to be related to the high incidence of cough in patients on ACE inhibitors and elevated bradykinin levels in the peripheral tissues, resulting in rapid fluid accumulation, are suspected of playing a key role in angioedema seen in a small number of patients taking ACE inhibitors. The association between ACE inhibitors and angioedema, first reported in the early 1980s, is now well recognized as a potentially serious but rare side effect of the drugs. Reactions occur with an incidence of about 0.1–0.5 % but the incidence in blacks (black Americans and Afro-Caribbeans) is about three times higher than in white populations. This, and the decreased antihypertensive response to ACE inhibitions in blacks, is thought to be due to decreased production of bradykinin and/or decreased vasodilation in response to the peptide vasodilator. In terms of the number and severity, ACE inhibitor-induced angioedema is said to account for 17 % of patients admitted for the treatment of angioedema and from 13 to 22 % of patients with this form of angioedema require airway intervention. In a 2008 study in Boston, USA, records of 220 patients who presented to five hospital emergency departments were reviewed. The frequency of ACE inhibitor-induced angioedema in all patients who presented with

angioedema was 30 %. The annual rate of visits for the drug-induced reaction was 0.7 per 10,000 emergency department visits. Eleven percent of the patients were admitted to intensive care and 18 % admitted to hospital for observation for a 24 h period. This study confirmed past experience and surveys concluding that ACE inhibitor-induced angioedema remains a rare condition, it represents a significant proportion of angioedema patients, and a subgroup of these patients require hospitalization for management of upper airway angioedema.

Figure 3.13 summarizes the individual reactions and the interactions and relationship between the renin–angiotensin system and the plasma kallikrein–kinin system. Activation by the enzyme prolylcarboxypeptidase (lysosomal carboxypeptidase) of the prekallikrein–high molecular weight kininogen complex on endothelial cells produces kallikrein which cleaves high (sometimes low) molecular weight kininogen liberating bradykinin. Bradykinin stimulates vasodilation and leads to the formation of nitric oxide (NO), superoxide and prostacyclin and the liberation of tissue plasminogen activator. Kallikrein in plasma and tissues also activates prorenin to renin, an aspartyl protease, which in turn activates angiotensinogen to angiotensin I. ACE converts the inactive decapeptide angiotensin I to the biologically active octapeptide angiotensin II which, like bradykinin, stimulates NO and superoxide formation as well as contributing to the elevation of blood pressure and local vasoconstriction and stimulating the release of plasminogen activator inhibitor I. ACE is also a major degrading enzyme for bradykinin (in fact, bradykinin is its preferred substrate over angiotensin I) producing the breakdown pentapeptide bradykinin(1–5) and in addition to its role in the formation of kallikrein, prolylcarboxypeptidase (with other enzymes) degrades angiotensin II to form angiotensin(1–7) which has vasodilatory and blood pressure-lowering activities. Overall then, stimulation of the bradykinin and angiotensin II receptors results in vasodilation and the production of NO and prostacyclin. Stimulation of the angiotensin I receptor leads to vasoconstriction and the elevation of blood pressure. It can be seen therefore, that the kallikrein–kinin and renin–angiotensin systems interact and are linked in a mutually dependent way.

Although it is beyond our requirements here, it should be pointed out that a homolog of ACE, angiotensin-converting enzyme 2 (ACE2), has recently been recognized. The two enzymes show different recognition of bradykinin. ACE2, a carboxypeptidase found mainly in the heart, kidney, and testis, does not degrade bradykinin but degrades des-Arg(9)-bradykinin at its carboxy-terminal amino acid and, unlike ACE which degrades angiotensin I by cleaving at the penultimate phenylalanine to produce angiotensin II [angiotensin(1–8)], ACE2 removes the carboxy-terminal leucine to form angiotensin(1–9). This peptide has been reported to enhance arachidonic acid release.

3.2.8.5.3 Angioedema Following Administration of Angiotensin II Receptor-Binding Inhibitors

Angiotensin II receptor blockers (ARBs) are primarily prescribed for high blood pressure but may also be used to treat heart attack, stroke, and congestive heart failure. Unlike ACE inhibitors, ARBs are not associated with cough. When first approved for the treatment of hypertension in 1995, ARBs were considered safe from the risk of edema and they are generally a safe alternative to ACE inhibitors, blocking the renin–angiotensin system more effectively than the latter drugs. By binding selectively to the angiotensin 1 receptors (AT1), ARBs do not affect ACE and therefore should not affect bradykinin levels but angioedema to ARBs does occur with an incidence ranging from about 0.1 to 0.4 %. From limited numbers examined, the risk of patients with angioedema to ACE inhibitors developing angioedema to an ARB is said to be from 2 to 17 % and for developing confirmed angioedema, 0–9.2 %. A review of 19 cases of ARB-induced angioedema found that 13 (68 %) had never received an ACE inhibitor. Angioedema has been reported after administration of losartan, candesartan, eprosartan, irbesartan, olmesartan medoxomil, and telmisartan. Cross-reactivity between ACE inhibitor- and ARB inhibitor-induced angioedema has been estimated to be from 3 to 8 %.

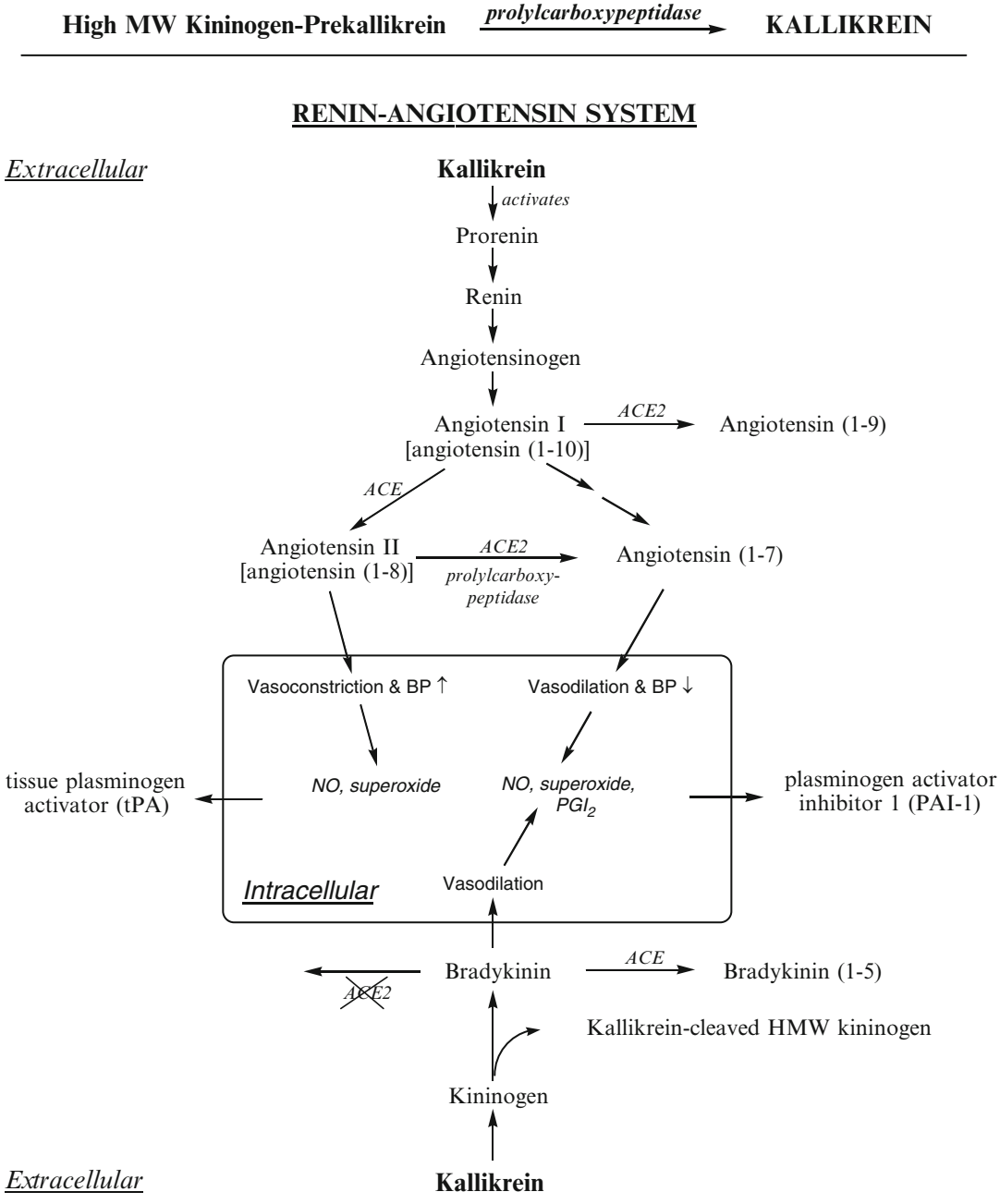


Fig. 3.13 Summary of the individual reactions involved in, and relationships between, the renin–angiotensin and plasma kallikrein–kinin systems. *ACE* angiotensin-converting enzyme, *BP* blood pressure, *MW* molecular weight, *NO* nitric oxide

Since angioedema to ACE inhibitors occurs as a result of increased bradykinin levels and ARBs are not known to affect these levels, the mechanism of ARB-induced angioedema is not understood. One suggested explanation is that unblocked angiotensin II receptors (AT2) are subjected to secondary stimulation by high levels of angiotensin II producing an increase in tissue bradykinin and hence angioedema. Another suggestion is an abnormality in the degradation of the active metabolite of bradykinin, des-Arg(9)-bradykinin.

3.3 The Allergen-Induced Late Phase Reaction

Exposure to allergen in the skin, lung, nose, or eye of atopic patients provokes an immediate or early response that is maximal at 20–30 min, resolves within about an hour, and is often followed 3–4 h after allergen challenge by a delayed reaction peaking at 6–12 h and subsiding by 24 h. The two reaction phases are well illustrated by an asthmatic response in the lungs of an allergic patient measured as falls in the peak expiratory flow rate (PEFR) following inhalation challenge with allergen. Figure 3.14 shows that the immediate response bottoms at about 30 min after allergen challenge before beginning to recover and climbing back over the next 30 min toward, but not reaching, the pre-challenged PEFR figure. Three to four hours after the initial allergen challenge there is a late phase response reflected in a pronounced fall in PEFR which reaches its maximum at 5–10 h. Thereafter there is a steady climb back to normal levels. The immediate response is caused by the release of histamine and some other preformed mediators from mast cells that have direct effects on blood vessels and smooth muscle. The initial release of the preformed mediators is supplemented over time by other powerful inflammatory agents including vasoactive agents that dilate blood vessels and produce edema, swelling, and pain. Figure 3.15 shows good examples of immediate and late phase cutaneous reactions. An immediate wheal and flare reaction and a late phase edematous response are seen 15 min and 6 h, respectively, following intradermal injection of antigen.

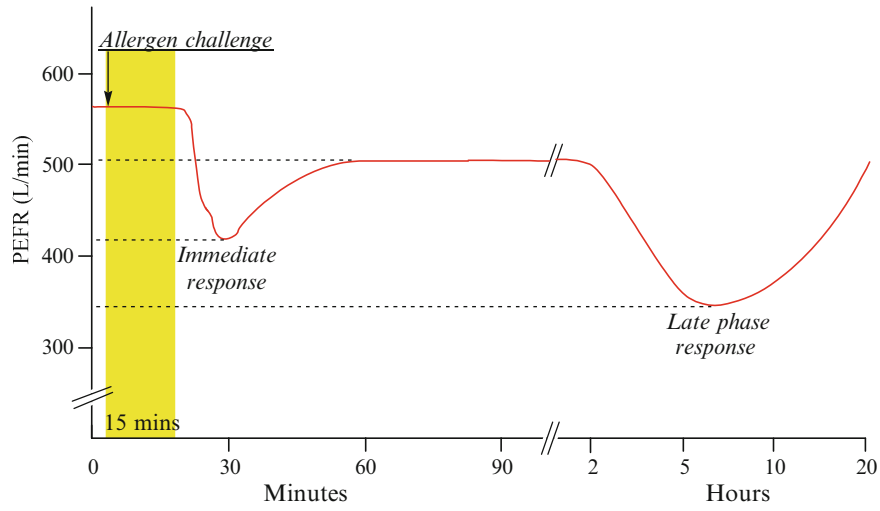


Fig. 3.14 A typical lung function result as measured by peak expiratory flow rates (PEFR) in an allergic patient following challenge with allergen. An immediate reaction at about 30 min is followed by a late phase response which reaches a maximum 5–10 h after allergen challenge

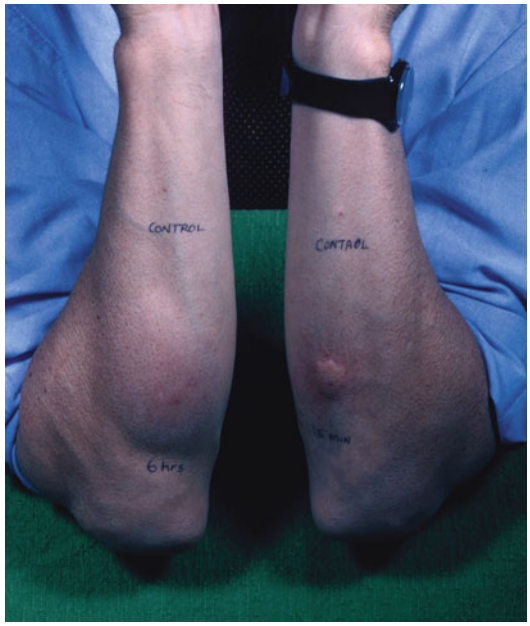


Fig. 3.15 An immediate wheal and flare cutaneous reaction in an allergic patient 15 min after intradermal injection of antigen shown alongside a late phase edematous response 6 h post injection (Photograph kindly provided by Professor S. R. Durham)

3.3.1 Early Studies: Implication of IgE Antibodies

Late reactions have been known for many years with initial published reports dating back nearly 100 years, but investigations of the underlying cellular events and mechanisms involved were not pursued in any systematic way until the late 1960s when Pepys and colleagues studied patients with allergic bronchopulmonary aspergillosis and extrinsic allergic alveoltis, also known as hypersensitivity pneumonitis. They found edema, perivascular cellular infiltration, deposition of complement and serum immunoglobulin precipitins to *Aspergillus fumigatus*, and a variety of other extracts from organisms and agents that cause allergic alveolitis and concluded that the late reactions were the result of an Arthus or type III reaction. Soon after, other investigators came to a different conclusion failing to consistently find precipitins and complement but strongly implicating IgE antibodies in the reactions by a variety of methods including direct demonstration by induction of immediate and late responses with affinity-purified allergen-specific IgE antibodies followed by allergenic challenge. Another important finding was the observation that lymphocytes were the predominant cell in the cellular infiltrates together with a significant number of eosinophils and basophils. It should be remembered, however, that the investigations implicating IgE antibodies in late reactions to *Bacillus subtilis* enzyme, ragweed pollen, and other inhalant allergens do not necessarily refute the conclusion of a type III Arthus reaction to *Aspergillus* species and other allergens responsible for hypersensitivity pneumonitis conditions such as bagassosis and farmer's, bird-fancier's, coffee worker's, malt worker's, and mushroom worker's lung. These are very different conditions to hypersensitivities to allergen sources such as ragweed pollen and dust mites and are characterized by different antigenic stimuli, symptoms of cough, dyspnea, pleurisy, fatigue, anorexia, and weight loss with interstitial granulomas and mononuclear and giant cells in the lungs.

3.3.2 Cellular Responses in the Late Phase Reaction and Comparison with the Delayed-Type Hypersensitivity Response

From undergraduates to clinicians and researchers, there has long been confusion over use of the terms "late" and "delayed" with the late phase of the immediate wheal and flare reaction sometimes being labeled and referred to as a delayed-type hypersensitivity reaction, a type IV reaction, or simply "DTH." There was therefore a need to research, compare, and contrast these reactions and this was done in an important study in which both responses were provoked in the same individuals and studied with the same panel of cell marker monoclonal antibodies together with immunohistologic methods. Skin biopsies from atopic individuals with late phase allergic skin reactions to intradermal challenge with grass

pollen or house dust mite were sectioned and examined for evidence of infiltration and activation of T cells and eosinophils. A substantial number of CD3+ and CD4+ cells but far fewer CD8+ cells were observed together with clearly different CD4+: CD8+ ratios in the sampled tissue and the peripheral blood. Infiltrated cells bearing receptors for IL-2 and evidence for IFN-γ secretion suggested that T cells had become activated. Activated eosinophils were also detected and there was a strong correlation between these cells and the numbers of CD4+ cells 24 h after the allergen challenge, suggesting that T cells participate in the late phase inflammatory reaction. In fact, about 50 % of cells infiltrating a late phase reaction site are T lymphocytes. It is therefore of interest to compare the late phase response with the delayed-type hypersensitivity reaction since it seems that T lymphocytes are important in both responses. In the comparison, grass pollen and house dust mite extracts were used to elicit late phase reactions while tuberculin challenge was used for delayed hypersensitivity responses. Both responses showed accumulation of CD4+ T cells, but overall the cells were more dispersed in denser accumulations and cells were still being recruited at 48 h in the delayed reactions. This is in contrast to the situation in late phase reactions where cell numbers usually plateau between 24 and 48 h. Other differences found were greater activation of eosinophils in late phase reactions, the detection of small numbers of these cells in atopics and non-atopics at 24 h but not at 48 h in delayed-type hypersensitivity, and greater T cell activation (demonstrated by expression of IL-2R) in the latter response. The release of inflammatory cytokines in both reactions was indicated by endothelial expression of HLA-DR. The allergen-induced late phase reaction then has features of a cell-mediated hypersensitivity response, but it shows some significant differences from the classical delayed hypersensitivity response in atopic subjects. The difference is perhaps best illustrated by the different cytokine profiles. Employment of labeled RNA probes for some cytokines showed that infiltrating cells from allergen-induced late phase cutaneous reactions have a Th2-like cytokine profile expressing mRNA for the cytokine gene cluster IL-3, IL-4, IL-5, and granulocyte macrophage colony-stimulating factor (GM-CSF). Cells from tuberculin biopsies on the other hand preferentially expressed mRNAs encoding for IL-2 and IFN-γ, that is, cells preferentially expressing a Th1 cytokine profile. Comparisons of the accumulation of inflammatory cells and cells expressing mRNA for different cytokines in late phase and delayed-type responses in the same subjects showed a relatively rapid (1–6 h) accumulation of T cells and granulocytes in the former case and a much longer accumulation time (24–48 h) for T cells, macrophages, and other cells expressing Th1-type cytokines in delayed hypersensitivity responses. At 48–96 h in the late phase response, some cells increasingly expressed Th1-type cytokines. This may be an indication of a classic delayed hypersensitivity response earlier masked by the IgE antibody-mediated reaction. Again, with the delayed-type response the distinction from the late phase response was not totally clear since a small number of cells in some individuals expressed mRNA encoding IL-4 and IL-5.

3.4 Drug-Induced Hypersensitivity and Immune Receptors

3.4.1 Background

An antibody response to a chemically reactive drug or hapten is said to occur after the drug–protein complex is recognized, processed, and presented as a drug–peptide conjugate to T cells that recognize the drug-modified peptide. A low molecular weight free, unconjugated drug is thought to remain unrecognized and not equipped to elicit an immune response. For drugs, however, immunological dogma is often found wanting on at least two counts. Firstly, despite the requirement that "small" molecular weight compounds or haptens (generally less than 1,000 kDa) need attachment to a macromolecular carrier to become immunogenic, many haptens or drugs that remain uncomplexed and apparently too

small do in fact elicit a clear immune response. Secondly, despite the pioneering findings of Landsteiner and other early immunochemists, and the conclusion that previous exposure to an allergen is a prerequisite for allergic sensitization and reactivity, the dogma of prior exposure does not always hold. Previous contact with a drug is not necessarily a prerequisite for a drug-induced immune hypersensitivity response. Although these inconsistencies were emphasized by the author and some other investigators over 20 years ago (see monograph on drug allergy, Further Reading), general acceptance of exceptions to the dogma has only recently been forthcoming.

3.4.2 Recognition and the Immune Response to Free, Unconjugated Drug

The implications for drug allergy from the basic and applied research on T cell recognition of haptens initiated just over 20 years ago, were perhaps best summed up by Weltzien who, in commenting on the advances, declared simply that the work may "contribute to a better understanding of what defines an antigen as an allergy-inducing allergen." Perhaps this will eventuate but since the fledgling discipline of hypersensitivity research moved beyond the embryonic stage in the early half of the twentieth century and matured over a 60-year period to provide impressive insights into the effector processes in the immediate allergic response, in particular the roles of IgE antibodies, mast cells, inflammatory mediators, and their receptor-controlled end organ responses, the long-standing question of what makes an allergen an allergen has remained obscure. Whether approaches utilizing T cell recognition of antigens in eliciting delayed hypersensitivity responses can soon provide the experimental and clinical opportunities to obtain the necessary insights and answers remains to be seen.

How then do small molecular weight, nonreactive chemicals such as many drugs stimulate IgE antibody production and provoke immune hypersensitivity reactions ranging from mild rashes to severe, life-threatening anaphylaxis? It must be understood that Landsteiner's studies on the sensitizing properties of some chemicals in the form of "small" molecules linked to a protein carrier constituted the initial investigations of contact hypersensitivity and the findings and interpretations from studies on protein conjugated chemicals should not automatically be used to explain all delayed reactions and certainly not IgE antibody-mediated type I reactions. An early clue to specific immune recognition of "small," unbound chemicals and hence drugs was the demonstration by Sinigaglia and his group of selective interaction of nickel (Ni) ions with an MHC-II-bound peptide. Ni-specific T lymphocyte clones from a patient with contact dermatitis to Ni responded to the metal ions when Ni salts were presented by APC in association with DRw11.1(5) molecules. Direct evidence that Ni was bound to the MHC-associated peptide was provided by NMR spectroscopy. These results, the first direct evidence of interaction between hapten and a MHC-bound peptide, not only demonstrated a model for Ni recognition by T cells from patients with Ni hypersensitivity but also indicated that a variety of chemically reactive groups, not only reactive metal ions, might attach to MHC-bound molecules to induce MHC-restricted responses to the conjugates. Further work with Ni hypersensitivity and the occupational lung disease berylliosis established that these conditions were MHC-II-linked CD4+ delayed-type hypersensitivity responses and that the high frequency of Ni-reactive T cells occurs by formation of reversible coordination complexes in which Ni interacts with the MHC and TCR via His81 of the HLA-DR α-chain and Tyr29 and Tyr94 of the CDR1α region of the TCR. This coordination complex of Ni ions directly linking the MHC peptide and TCR is similar to the action of a weak superantigen. In extending the studies on Ni to investigations on the T cell recognition of haptens, Weltzien and others have shown, somewhat surprisingly to some, that MHC-restricted hapten-specific T cell receptors react to hapten–peptide conjugates within the MHC peptide-binding groove. This opened up a new approach for studying the molecular mechanisms underlying hapten recognition by T cells.

3.4.3 Abacavir and the MHC-Presented Altered Peptide Model of Drug Hypersensitivity

More recently, some interesting HLA associations in drug hypersensitivities have been reported. A strong association of hypersensitivity to the guanosine-related pro-drug and reverse-transcriptase inhibitor abacavir was found with the well-defined 57.1 MHC haplotype encoding the MHC class I allotype HLA-B*57:01 (see Sect. 1.3). Multi-organ reactions to abacavir, termed abacavir hypersensitivity syndrome or AHS, manifests as fever, rash, malaise, nausea, and diarrhea. It occurs in approximately 2–8 % of patients with human immunodeficiency virus-1 (HIV-1) infection and can be severe enough to cause death in some rechallenged patients. Abacavir-specific CD8+ T cells secrete TNF and IFNγ and are cytotoxic to abacavir-APCs. In a 2008 study, implication of the fine-structural specificity of the 6-cyclopropylamino group of abacavir as a possible reactive site in the HLA-restricted CD8+ T cell response was demonstrated by lack of recognition of the abacavir structural analogs carbovir, didanosine, and guanosine by abacavir-reactive T cells (Fig. 3.16). Specificity of the interaction was further mapped to the F pocket (one of six, termed A–F), of the MHC-1 antigen-binding cleft where it was thought that abacavir, or a metabolite, binds to one or more self-peptides. At that stage, whether the binding was covalent or not had yet to be determined. It was predicted that the demonstration that AHS is an MHC-I-restricted cellular hypersensitivity response mediated by CD8+ T cells might prove to be a forerunner for our better understanding of the basis of immune receptor recognition in drug hypersensitivities and, more specifically, for elucidating the pathogenesis of some of the life-threatening drug-induced systemic reactions such as toxic epidermal necrolysis (TEN) and Stevens–Johnson syndrome (SJS).

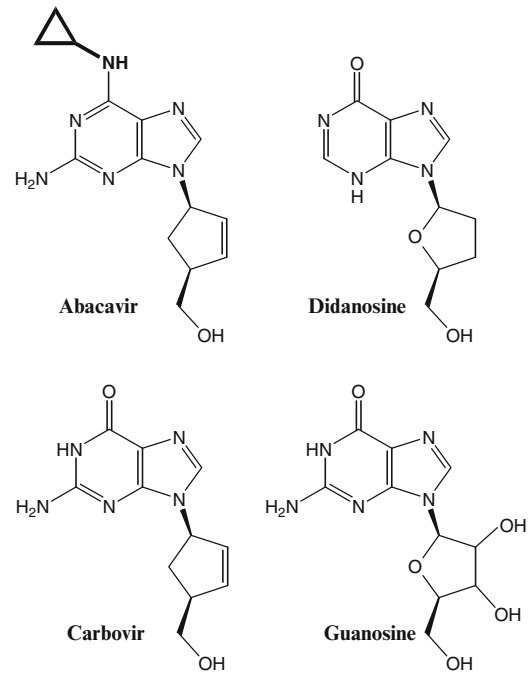


Fig. 3.16 Structures of abacavir and three purine analogs didanosine, carbovir, and guanosine. The 6-cyclopropylamino group of abacavir is highlighted

Right at the time of the completion of this monograph, newly published results explaining the molecular basis of AHS give every indication of having profound implications for understanding the origins and general molecular processes of autoimmunity. AHS is mediated by abacavir-specific activation of cytotoxic CD8+ T cells that require HLA-B*57:01 antigen-presenting cells, but abacavir-specific T cells are not activated by cells expressing the closely related allotypes HLA-B*57:02, HLA-B*57:03, and HLA-B*58:01, each of which is insensitive to abacavir and not linked to AHS. Two amino acid residues, Asp114 and Ser116, distinguish HLA-B*57:01 from the abacavir-insensitive alleles and abacavir reacts with these two amino acid residues. In extending the finding that the abacavir-HLA-B*57:01 association results from specific binding of the drug to the HLA F pocket, amino acid sequences of a large number of HLA-B*57:01- and HLA-B*57:03-bound peptides from untreated and abacavir-treated cell lines were determined. Abacavir-treated HLA-B*57:01 cells, but not treated HLA-B*57:03 cells, contained unmodified drug but no metabolites

indicating that abacavir bound non-covalently and specifically with HLA-B*57:01. Up to 25 % of the peptides bound to HLA-B*57:01 following treatment with abacavir proved to be different to those before treatment but a change was not seen with peptides bound to HLA-B*57:03 or HLA-B*58:03. These results again suggested that abacavir binds specifically to the antigen-binding cleft of HLA-B*57:01 and this alters the repertoire of self-peptides bound by HLA-B*57:01 but not the repertoire bound by the other HLA alleles. Sequences of peptides that bind HLA-B*57:01 contained Trp → Phe at the C terminus (PΩ) but for HLA-B*57:03 PΩ was reversed, i.e., Phe → Trp. After abacavir treatment, an increased number of peptides with Ile or Leu at PΩ bound HLA-B*57:01. In another recent study, peptides eluted from an HLA-B*57:01 single allele-transfected cell line treated or not treated with abacavir were analyzed. A significant number of peptides with Val at the C-terminus were identified in the presence of abacavir but no peptides with Val at the C-terminus were found in untreated cells. Significant numbers of peptides with terminal Ile and fewer peptides with Trp and Phe also occurred in the presence of abacavir. Taken together, the results of the abacavir–HLA binding studies indicate that the drug positions itself at the bottom of the antigen-binding cleft extending, via the cyclopropyl moiety (Fig. 3.16), into the F pocket and changing the shape of the cleft. This results in preferred binding of smaller amino acids, an alteration in the repertoire of self-peptides that bind HLA-B*57:01, and a T cell response to self-proteins presented only in the presence of abacavir. Extension of this investigative approach to the antiepileptic carbamazepine, a drug strongly associated with HLA-B*15:02 (see below), showed that the drug binds to this allotype and, again, an altered repertoire of presented self-peptides results. This raises the possibility that antigen-presenting molecules may be susceptible to modulation by drugs (and perhaps even toxins, environmental chemicals, etc.) causing altered T cell immunity. If this is a general mechanism, investigations of associations of other drug hypersensitivities with antigen-presenting molecules may reveal further fascinating insights into some poorly understood, unpredictable, and potentially life-threatening adverse drug reactions and ultimately lead to a better understanding of the immunopathogenesis of autoimmunity, infectious diseases, and cancer.

3.4.4 Carbamazepine and Other HLA-Drug Hypersensitivity Associations

In addition to the associations of HLA-B*57:01 with abacavir hypersensitivity and flucloxacillin-induced liver injury (Sect. 5.1.10), HLA-DRB1*15:01 with lumiracoxib-induced hepatotoxicity, and HLA-B*58:01 with allopurinol-induced SJS (see below), HLA-B*15:02 is strongly associated with carbamazepine-induced SJS and TEN. As mentioned in Sect. 3.4.3 above, the generality of the abacavir–HLA binding results was tested in a preliminary way in an examination of the well-established strong association between HLA-B*15:02 and carbamazepine-induced SJS/TEN in Asian populations. A non-covalent association between carbamazepine and HLA-B*15:02 was established by purifying HLA-B*15:02–peptide complexes and sequencing of bound peptides. This revealed a preference for smaller amino acids at key positions and significant increases in the presence of some hydrophobic residues. Comparisons with HLA-B*15:01 show that this allele is not associated with carbamazepine-induced SJS and an important difference between HLA-B*1502 and HLA-B*15:01 is at position 156 (Leu for the former, Trp for the latter) near where the drug is thought to bind in HLA-B*15:02.

The carbamazepine–HLA-B*15:02 interaction has also recently been used by S-I Hung and collaborators in Taiwan as a model for the study of the pathologic role of HLA in delayed-type drug hypersensitivity. No intracellular metabolism or antigen processing was detected in the interaction between carbamazepine and HLA-B*15:02 in patients with the bullous skin

conditions and surface plasmon resonance assays showed that HLA-B*15:02, but not other HLA-B recombinant proteins, directly binds carbamazepine and the structurally related carbamazepine 10,11-epoxide. For drug presentation and activation of cytotoxic T lymphocytes, endogenous peptides in the antigen-binding groove were shown to be necessary. This is in contrast to abacavir which binds to HLA-B*57:01 without peptide loading. Modeling suggested that the Arg62 side chain, located in the B pocket of the HLA-B*15:02 protein, was the most likely binding site for carbamazepine by forming a hydrogen bond with the ketone of its 5-carboxamide group on the tricyclic ring. Specific recognition of this group was supported by results obtained with selected structural analogs.

Allopurinol is an important treatment for hyperuricemia-related diseases, being used to lower uric acid in gout, kidney stones, and Lesch–Nyhan syndrome. Unfortunately, the drug is also a frequent cause of adverse drug reactions, accounting, it is said, for up to 5 % of severe cutaneous adverse reactions. Reactions include drug hypersensitivity syndrome, SJS, and TEN. In a Taiwanese study designed to identify genetic markers for allopurinol cutaneous reactions, the HLA-B*58:01 allele was identified in 100 % of 51 patients with allopurinol-induced serious reactions but in only 15 % (20) of 135 tolerant patients. These results indicate that in Han Chinese, allopurinol is strongly associated with HLA-B*58:01 and this allele is an important genetic risk factor for the serious cutaneous reactions with systemic symptoms.

The NSAID and phenylbutazone derivative, feprazone, was found to be associated with HLA-B22 in a Scandinavian study—93 % of patients with a fixed drug eruption caused by the drug were HLA-B22 positive but no patients with fixed drug eruptions to other drugs were positive to HLA-B22 and this allele was found in 4 % of healthy controls. However, a number of factors, including the absence of HLA-B22 in 7 % of the Scandinavian patients with feprazone-induced fixed drug eruption, need further scrutiny before feprazone-HLA-B22 can be taken as a diagnostic marker. At the least, more extensive population studies are needed.

Other drug hypersensitivities or intolerances thought or claimed to be associated with HLA class I and/or class II alleles include trimethoprim-sulfamethoxazole with HLA-A30, aspirin with a number of different haplotypes (see Sect. 9.5.5), hydralazine-induced systemic lupus erythematosus with HLA-DRw4, and nevirapine hypersensitivities with a surprising and confusing array of associations. Again, these findings highlight the need for extensive phenotyping studies and investigations in much larger populations.

3.4.5 The Question of Direct Drug Activation of T Cells Without Involvement of a Specific Peptide

Drawing on earlier speculations on the absence of prior sensitization in many drug reactions and the seminal studies of MHC-restricted metal ion and drug hypersensitivities mediated by T cell activation, others have suggested some modifications to the possible cellular and drug interactions involved in drug-specific recognition by cells of the immune system. A number of observations including the prevention of T cell activation after removal of drug by washing, rapid calcium influx into T cells after exposure to drug, and the fact that glutaraldehyde-fixed APC can still present drug have led to the suggestion that T cells rather than APCs recognize free, unprocessed parent drug in allergic individuals. Proponents of the so-called p–i concept (derived from the proposed direct pharmacological interaction of drugs with immune receptors) state that "drugs bind specifically and reversibly to some of the highly variable antigen-specific TCR in a direct way, instead of covalently modifying the MHC-peptide complex." Direct and in-depth experimental findings of the sort presented in the Ni, abacavir, and carbamazepine/HLA-B*15:02 investigations to support this hypothesis are lacking, and with, for example, abacavir bound at the bottom of the HLA-binding groove, it is difficult to see how the drug can directly contact, or by itself directly influence, the T cell receptor. Recently the proposed model restricted to T cell binding appears to have been modified to

acknowledge and to try and accommodate the association and presentation of some drugs, or drugs with peptide, in the MHC peptide-binding groove. Given the apparent unequivocal definition of direct drug binding to the TCR without modification of the MHC–peptide complex, it is difficult to see how this accommodation can be achieved.

3.5 Desensitization of Drug-Allergic Patients

Only immediate type hypersensitivity drug reactions involving IgE antibodies and/or a mast cell-mediated mechanism are considered eligible for desensitization.

An adverse reaction to a drug can be major problem in efforts to achieve successful treatment for common and important diseases including infections, arthritides, allergies, and malignancies. Adverse drug reactions occur frequently, and as the number, chemical nature, and novel pharmacological actions of registered drugs continue to increase, such reactions can seriously interrupt therapy and leave patients with less than optimal treatments. Rapid drug desensitization (RDD) can provide an effective and safe means to continue vital therapies while minimizing or avoiding the previously disruptive impediment. The aim of desensitization is to administer increasing amounts of drug in an incremental and stepwise manner while at the same time avoiding or minimizing life-threatening, or even lesser adverse, symptoms. When successful, the procedure induces temporary tolerance to the drug allowing treatment to continue with optimal dosage.

In considering possible mechanisms leading to rapid desensitization to a drug, the mast cell and possibly the basophil appear to be the cells most likely to be involved. In drug reactions involving complementary IgE antibodies, RDD appears to result in the mast cells becoming temporarily tolerant to the drug. A convincing explanation of how RDD tolerizes mast cells or interferes with their activation is lacking and the subject is inadequately understood and in need of further investigation of possible mechanisms. One current investigative approach involves the delivery of increasing quantities of antigen at fixed time intervals to mouse bone marrow mast cells in vitro together with the monitoring of granule release by detection of β-hexosaminidase and the metabolism of prostaglandins and leukotrienes. Both of these indicators were inhibited by desensitization and this was achieved by incremental increases in dosage. Importantly, the presence of antigen was necessary for desensitization—as long as antigen was maintained, and desensitization was maintained. Mast cells desensitized to dinitrophenol did not release preformed and de novo synthesized mediators such as TNF and IL-6. This may help to explain why desensitized patients are not at risk of a delayed reaction. Experiments in which mast cells were sensitized to dinitrophenol and ovalbumin showed that ovalbumin-desensitized cells responded fully to dinitrophenol, proving antigen specificity and that signaling transduction pathways have not been impaired during desensitization. Furthermore, FcεRI-bound antigen-specific IgE molecules did not disappear from the cell surface during desensitization after becoming bound to small doses of antigen. These results are reassuring in that they support both the proposed inhibition of the mast cell response and the basis for the RDD procedures currently used. Over many years, a number of other mechanisms have been proposed to explain the state of clinical tolerance resulting from the practice of desensitization. The list includes the formation of IgG blocking antibodies, consumption or blocking of the drug-reactive IgE antibodies by the gradually increasing quantities of administered drug, tachyphylaxis or depletion of the released mediators, hapten inhibition by monovalent penicillin–protein conjugates, and desensitization of mast cells and basophils by gradually increasing quantities of multivalent drug–carrier complex. It must be concluded, however, that few truly revealing insights into the mechanisms underlying mast cell tolerance or hypo-responsiveness have been obtained so far.

Note that desensitization to a drug does not result in long-term tolerance to the adverse effects of the drug and this therefore means that

patients need the desensitization procedure to be repeated each time they are again exposed to the drug. However, if the medication is maintained, for example, by daily dosage with pharmacologically active levels, the desensitization state can be maintained.

3.6 Delayed-Type (Type IV) Hypersensitivity Drug Reactions

Unlike types I, II, and III hypersensitivities, which are mediated by antibodies, delayed or cell-mediated hypersensitivity, classified as type IV, is mediated by antigen-specific effector T cells and this means that the hypersensitivity response can be transferred by purified T cells or a cloned T cell line. Again in contrast to an immediate reaction, a delayed-type hypersensitivity reaction develops over a period of 24–72 h. Delayed hypersensitivity responses have been used for many years to assess patients' cell-mediated immunity by the induction of induration and erythema 48–72 h after intradermal injection of so-called "recall" antigens from *Mycobacterium tuberculosis*, *Candida* and *Trichophyton* species, and tetanus toxoid.

3.6.1 The Cellular Basis of Type IV Hypersensitivity Cutaneous Drug Reactions

Delayed-type hypersensitivity reactions in the skin provoked by systemic drug administration usually occur 7–10 days after the commencement of therapy. Drug-induced skin reactions manifest mainly as exanthemas, mediated by CD4+ and CD8+ CD3+ T cells in the dermis and epidermis. Antimicrobial drugs, NSAIDs, and some analgesic drugs are the biggest causes of drug-induced adverse cutaneous reactions but a variety of other drugs including anticonvulsants (e.g., carbamazepine), local anesthetics (lidocaine), cardiovascular drugs (procainamide), and antipsychotics (clozapine) are well known to cause reactions. For most proteins and hapten–protein conjugates, processed antigen is presented to CD4+ T cells via the MHC class II molecules on antigen-presenting cells. The cells involved in many type IV responses such as contact hypersensitivity and psoriasis are Th1 and CD8+ cytotoxic T cells while in a condition such as allergic contact dermatitis, CD4+ or CD8+ T cells can be activated depending on the antigen processing pathway. In general, CD4+ T cell activation seems to mediate maculopapular and eczematous drug hypersensitivities while CD8+ T cell activation produces the more severe skin reactions involving bullous manifestation.

In a hypersensitivity reaction in the skin such as allergic contact dermatitis, there are two phases of the hypersensitivity response, sensitization (or initiation or induction) and elicitation. In the sensitization phase following drug administration, free drug or drug bound to a protein carrier reaches the skin where it encounters keratinocytes, present in great numbers and thought to play a major role in the initiation of skin sensitization. As well as facilitating the formation of biologically active haptens and hapten binding to protein, keratinocytes release chemotactic factors CXCL8, CXCL9, CXCL10, and CXCL11 and adhesion molecules (e.g., ICAM-1) on exposure to sensitizing agents. These chemotactic factors attract more cells to the active skin sites, thus increasing local immune activity. Sensitization proceeds with drug–carrier complex being taken up by immature Langerhans' and dendritic cells. These migrate to the draining lymph node and, with the stimulus provided by co-stimulatory molecules, become T cell-activating cells. Processed antigen is expressed as a drug–peptide complex in association with MHC class I and II molecules on the surface of the mature antigen-presenting cells for presentation to CD8+ and CD4+ T cells, respectively. Dendritic cells, Langerhans' cells, and skin macrophages express both MHC (class I and II) molecules and can activate CD4+ as well as CD8+ T cells. Keratinocytes are also important in the elicitation phase and can present antigen via MHC I and MHC II molecules. T cells are activated, undergo clonal expansion, and give rise to cells with different memory and effector

functions, that is, Th1, Th2, or Th17 CD4+ or CD8+ T cells. By the time of the elicitation phase, T cells have gained access to the skin and following reexposure to the drug, skin symptoms occur within about 48 h. Hapten-specific T cells recognize the hapten-peptide presented by dendritic cells and keratinocytes and the resultant activated T cells produce Th1 and Th17 cytokines such as IFN-γ, IL-12, IL-17, and IL-23. Note that although Langerhans' cells have long been considered to be the classical cell net to trap, process, transport, and present antigen to T lymphocytes, evidence, including mice lacking Langerhans' cells, has shown that dendritic cells can act in their place if Langerhans' cells are absent or functionally affected. The nature of the antigen, that is, the sensitizing drug or drug conjugate, seems to determine which MHC molecule is involved in antigen presentation. Extracellular antigens (for example, contact allergens) are generally presented via MHC class II molecules and intracellular antigens (for example, drug–protein conjugates formed intracellularly) via MHC class I. Presentation, for example, of the contact sensitizing agent nitrobenzene sulfonic acid, appears to be by MHC II molecules.

With the involvement of the co-stimulatory B7-CD28 interaction, T cells are activated and memory T cells can be found in the dermis. Other co-stimulatory molecules have also been identified including OX-40-OX-40L, PD-1-PD-L1 and PD-L2, RANK-RANKL, and CD40-CD40L (CD154). The receptor OX-40, also known as CD134, and its ligand OX-40L are seen as secondary co-stimulatory molecules expressed after T cell activation and important in maintaining T cell memory. RANKL, involved with dendritic cell maturation, belongs to the TNF cytokine family while PD-1 and its ligands, belonging to the B7 family, negatively regulate T cell responses. Binding of the co-stimulatory molecule CD-40 on antigen-presenting cells to its ligand CD40L activates these cells. During subsequent exposure of the memory T cells to the sensitizing antigen, clonal expansion of the activated T cells occurs and this ultimately results in T cell-mediated inflammation and cell damage in the skin. Activation and proliferation of memory T cells in the dermis release chemokines and inflammatory cytokines such as IFN-γ and tumor necrosis factor-α/β (hereafter referred to as TNF) that recruit macrophages to the site. Presentation of antigen by the newly arrived macrophages has the affect of further amplifying the response. The released chemokines and cytokines increase the permeability of blood vessels leading to local swelling and induce the expression of vascular adhesion molecules. IFN-γ is the key cytokine and it plays a dominant part in delayed hypersensitivity, upregulating T cell activation markers and MHC molecules, and aiding Th1 while suppressing Th2 cell differentiation. TNF also has a central role in delayed hypersensitivity, inducing chemokine production, upregulating expression of adhesion molecules, and promoting the influx of inflammatory cells. CD4+ and CD8+ T cell-mediated cytotoxicity of skin cells presenting drug can result from interaction with Fas/FasL, release of the cytolytic protein perforin and the serine protease granzyme B from cytotoxic T lymphocytes, and release of granulysin from cytotoxic CD8+ T cells. In response to inflammatory agents released by T cells, skin cells can in turn contribute to inflammation by releasing their own spectrum of cytokines and chemokines that stimulates further leukocyte recruitment into the skin. As understanding of the complexities of the mechanisms of the many processes that make up delayed-type hypersensitivity responses increases, two other agents, Il-12 and osteopontin, are attracting the interest of researchers. IL-12, produced mainly by antigen-presenting cells, aids the proliferation and differentiation of Th1 cells, augments IFN-γ production by these cells, and enhances NK and CD8+ T cell cytotoxicity. Osteopontin (also known as ETA, early T lymphocyte activation-1), a phosphoglycoprotein with cytokine and chemotactic functions, has a Th2 suppressive effect augmenting Th1-mediated allergy such as allergic contact dermatitis and supporting dendritic cell migration and Il-12 expression and secretion. Discussion of osteopontin's role in allergic contact dermatitis is continued briefly below in Sect. 3.6.3.1.

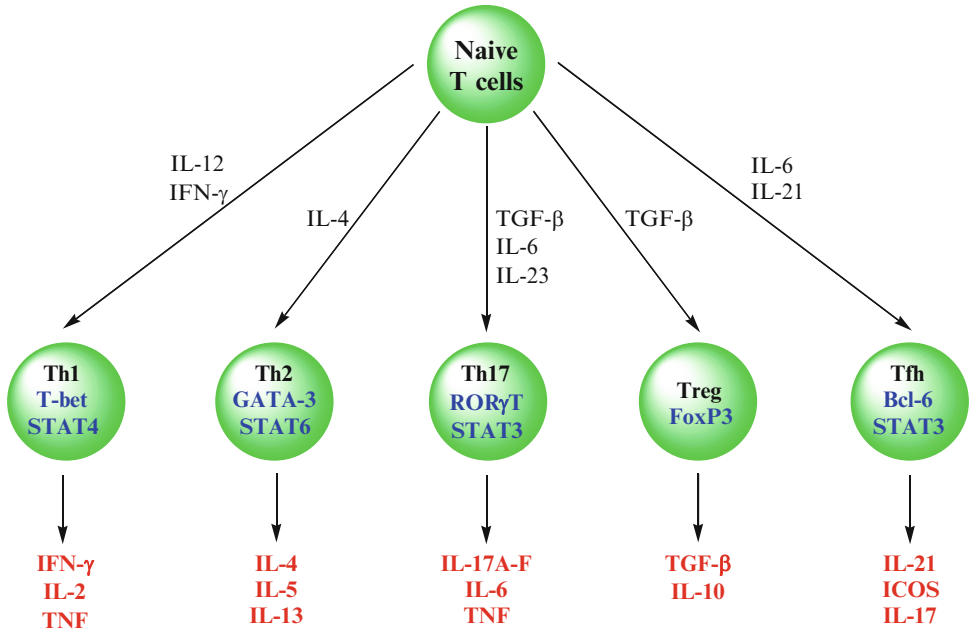


Fig. 3.17 Naive T cells, under the influence of cytokines produced by other immune cells, undergo activation and polarization to distinct Th subsets. Each subset displays a distinct cytokine secretion profile resulting in different effector functions, e.g., Th1 cells activate macrophages; Th2 cells promote allergic responses and immune responses to parasites; Th17 cells promote inflammation by helping to recruit neutrophils and Treg cells exert a number of inhibitory actions via cell contact. A more recently identified CD4 T cell subset, termed follicular helper cells (Tfh), provide a helper function to B cells

3.6.2 T Helper Cell Responses and Th17

As discussed, naive T cells differentiate into Th1 or Th2 cells during activation induced by interaction with dendritic cells with toll-like pattern-recognition receptors that detect the nature of the antigen. This results in IL-12 production, the involvement of transcription factors T-bet, STAT4, or STAT1 within the T cell, the induction of Th1 differentiation, and production of IFN-γ. Th2 differentiation is the result of IL-4 cytokine and GATA-3 and STAT6 transcription factor involvement that drives production of IL-4, IL-5, and IL-13. TGF-β and the transcription factor FoxP3 results in the T_{reg} T cell subset that secretes TGF-β (Fig. 3.17). After definition of the Th1 and Th2 subsets more than 20 years ago, relatively recent research has revealed a new class of T effector cells Th17, induced from naive T cells by the cytokines TGF-β and IL-6 and enhanced by IL-23, a cytokine produced by keratinocytes, Langerhans' cells, dendritic cells, and macrophages. Th17 cells are characterized by expression of distinct transcription factors RORγT, STAT3, and IRF-4 and the production of pro-inflammatory molecules of the IL-17 family comprising IL-17A, B, C, D, E, and F (Fig. 3.17). IL-17A gives rise to tissue inflammation by producing pro-inflammatory cytokines IL-6 and TNF and chemokines CCL2 (monocyte chemotactic protein-1 or MCP-1), CXCL1, and CXCL2 that activate macrophages and granulocytes. IL-25 and IL-27 negatively regulate Th17 cells while Th17 polarization is inhibited by IL-2, IL-4 (induces Th2), and IFN-γ (induces Th1).

Another more recently identified CD4 T cell subset, termed follicular helper cells (Tfh), provides a helper function to B cells. Tfh cells are distinguished from Th1 and Th2 cells by expression of the chemokine CXCR5, their association with B cell follicles, and their B cell helper function.

They produce ICOS (inducible T cell co-stimulator) and IL-21, a cytokine that stimulates B cells to differentiate into antibody-forming cells. This cytokine is particularly interesting for those concerned with understanding allergic mechanisms and the treatment of immediate-type allergies. IL-21 knockout mice express higher levels of IgE than normal mice and, in fact, IL-21 has already been used to attenuate allergic responses by reducing both IgE and inflammatory cytokine production in mouse models for rhinitis and peanut allergy.

3.6.3 Delayed Cutaneous Adverse Drug Reactions

For the first episode, these reactions generally begin 7–21 days after contact with drug. Subsequent reactions begin 1 or 2 days after reexposure. Specificity is usually demonstrated by oral challenge with small doses of the culprit drug, a positive patch test or intradermal test generally read after a delay of at least 48 h, and perhaps a positive in vitro lymphocyte proliferation assay. Activated T cells are found in the skin and in some cases T cell lines and clones can be isolated from blood and/or skin sites. Distinct subsets of T cells with their accompanying profile of cytokines and chemokines promote the inflammatory and cytotoxic responses seen in the different clinical patterns characteristic of the various drug-induced adverse cutaneous hypersensitivities. Individual hypersensitivity eruptions are essentially the result of overlapping cytokine actions with one or a few such actions dominant and characteristic of the delayed drug hypersensitivity phenotypic pattern. This, and the lack of histological and immunocytochemical criteria, has consequences for the diagnosis of drug-induced skin reactions where considerable effort is needed in the development of reliable and specific tests that can be easily undertaken (see Chap. 4). Although the mechanisms underlying the different drug-related skin eruptions with an immunological pathogenesis are still far from precisely defined, summaries of the progress are set out below.

3.6.3.1 Allergic Contact Dermatitis

For a description of allergic contact dermatitis, see Sect. 2.2.4.1 and Figs 2.4 and 2.5. Not all contact dermatitis has an immune basis; some irritants such as organic solvents, highly alkaline drain cleaners, and sodium lauryl sulfate and some phototoxins like the psoralens, paradoxically used for the treatment of psoriasis, eczema, and vitiligo, may also provoke reactions. Common causes of allergic contact dermatitis include Ni metal, chromium, balsam of Peru, and Toxicodendron plants, for example, poison ivy, poison oak, and poison sumac. Causative agents tend to be reactive small molecules or haptens of less than 1,000 Da that can easily penetrate the skin barrier and form covalent adducts with cutaneous proteins. Allergic contact dermatitis is regarded as a Th1 and CD8+ T cell-mediated disease and Ni allergy (see also Sect. 3.4.2), which involves activation of HLA-restricted, skin-homing Ni-specific T cells by antigen-presenting cells, is perhaps its best-known commonly occurring form. Both sensitization and skin reactions to Ni are thought to be mediated by CD4+ and CD8+ effector T cells producing IFN-γ. During sensitization when no clinical symptoms are apparent, mature Langerhans' cells originating from skin sub-layers present Ni-peptide-MHC complex to T cells in local lymph nodes. Upon rechallenge with Ni, the effector phase of allergic contact dermatitis is activated to produce cutaneous infiltration of Ni-specific and CCR4-positive T cells. Ni-specific cytotoxic CD8+ T cells release inflammatory cytokines that produce the characteristic skin lesions at the site of Ni sensitization (Chap. 2, Fig. 2.4). The T cell cytokine IL-17 can be found in the skin of patients with allergic contact dermatitis. Some Ni-specific CD4+ T cell clones isolated from the blood of allergic contact dermatitis patients express this cytokine which regulates the expression of adhesion molecules by keratinocytes and the synthesis and release of the chemokines IL-8 and RANTES. IL-17 has been shown to be locally released by Ni-specific Th0, Th1, and Th2 lymphocytes in the skin of patients with allergic contact dermatitis where it amplifies reactions and modulates the pro-inflammatory action of

keratinocytes by acting together with IFN-γ and IL-4. There still seems much to learn about the role of IL-17 in allergic contact dermatitis but already the importance of this cytokine in the pathomechanism underlying the condition is apparent.

The phosphoglycoprotein *osteopontin* is expressed by a number of different immune cells including effector T cells and keratinocytes in allergic contact dermatitis. The molecule is expressed in secreted and intracellular form, it enhances Th1 and Th17 immunity, and protects against apoptosis. Experiments in mice have shown that T cell clones secreting low levels of IFN-γ may compensate by secreting high levels of osteopontin which, in turn, down-modulates T cell IL-4 expression. In allergic contact dermatitis, secretion of IFN-γ by effector T cells induces osteopontin in keratinocytes which ultimately results in the attraction of inflammatory cells. The demonstrations that osteopontin-null mice display a reduced inflammatory response in contact hypersensitivity and anti-osteopontin antibodies partly suppresses established chronic contact sensitivity suggest that osteopontin may be a promising therapeutic target in allergic contact dermatitis.

3.6.3.2 Psoriasis

Clinical aspects of psoriasis are presented in Sect. 2.2.4.2 and Fig. 2.6. The classification of T cells into Th1 and Th2 cells, essentially on the basis of their defining cytokines IFN-γ and IL-4, respectively, and the fairly recent identification of a new type of T cell, Th17, together with the realization of its importance in inflammation, has led to the reexamination of many diseases previously considered to be solely Th1 or Th2 mediated. So far, in murine models at least, some diseases, previously thought to be Th1-mediated responses, have been found to involve both Th1 and Th17 cells. Th17 cells produce IL-17, TNF, IL-6, IL-21, and IL-22 which are upregulated during inflammatory disorders and which produce thickening of mouse epidermis suggesting a role in psoriatic inflammation. Other findings suggestive of a role for Th17 cells in psoriasis include the reduction of levels of IL-17 and IL-22 in the serum of patients whose psoriasis had been cleared by treatment with the TNF inhibitor etanercept; enhanced expression of IL-23 in patients with psoriatic lesions; and the demonstration of IL-17 mRNA in psoriatic lesions. Analysis of psoriatic skin lesions and peripheral blood for the presence of IL-17-producing cells revealed Th17 cells localized in the lesions and the dermis. In addition, IL-17 mRNA expression returned to normal with cyclosporin therapy and IL-22 mRNA expression moved in parallel with IL-17 changes, suggesting that both Th1 and Th17 cells are active in the inflammatory stages of psoriasis. Following the demonstration that in addition to Th1 cells producing IFN-γ, CD4+ T lymphocytes producing IL-17 were also important in the pathogenesis of psoriasis, attention turned to the possible importance of IL-17-producing CD8+ cells known to be present in psoriatic plaque. Investigations showed that CD8+ IL-17+ cells produced the Th1-related cytokines IFN-γ and TNF as well as the Th17 cytokines IL-17, IL-21, IL-22, and upregulation of the transcription factor RORC. These results showing some common properties between CD8+ IL-17+ T cells and Th17 cells and the intriguing finding that CD8+ cells, unlike Th17 cells, can also make IFN-γ and TNF may prove significant in fully elucidating the pathogenesis of psoriasis.

Currently, the broad understanding of the events and mechanisms leading to psoriasis is as follows. Antigen-presenting cells, probably Langerhans' cells, in the skin migrate to regional lymph nodes where they interact with T cells. The nature of the presented antigen is not known but co-stimulatory factors from the antigen-presenting cell are believed to be intercellular adhesion molecule 1 (ICAM-1, CD54) and lymphocyte function-associated antigen 3 (LFA-3, CD58). These molecules interact with their complementary receptors on the T cell, lymphocyte function-associated antigen 1 (LFA-1, integrin), and LFA-2 (CD2), respectively. Activated T cells return to the skin where local effects in the dermis and epidermis of released pro-inflammatory cytokines such as TNF produce the inflammation and epidermal hyper-proliferation seen in psoriasis.

3.6.3.3 Maculopapular Exanthema

A case of maculopapular exanthema induced by amoxicillin with lesions on the trunk and hands is shown in Fig. 2.7 together with a clinical description in Sect. 2.2.4.3. Lymphocytes (CLA+, CD3+, DR+, CD25+) expressing adhesion molecules are attracted from the blood by adhesion molecules expressed by endothelial cells and keratinocytes and by chemokines such as CCL27 (also called cutaneous T cell-attracting chemokine CTACK).

Both CD4+ and CD8+ T cells are found in the skin and blood of patients with maculopapular exanthema, but findings on the relative importance of these cells differ with some authors stating that CD4+ cells predominate and inflict cell damage by expressing high levels of perforin and granzyme B while CD8+ cells are found mainly in the epidermis. Other results have shown that CD8+ cells predominate in acute lesions of the epidermis and are the major drug-specific cytotoxic cell found in the blood of most patients with penicillin-induced maculopapular exanthema. Examination of cellular infiltration in the skin of patients during patch testing demonstrated rapid recruitment of CD8+ cells after skin contact with drug and before appearance of other cells particularly CD4+ T cells. Both type 1 and type 2 cytokines are produced; IFN-γ (type 1) activates dendritic cells and keratinocytes; IL-5 (type 2) together with eotaxin (CCL11) recruits and activates eosinophils. Other chemokines including CCL20, CXCL9, and CXCL10 appear to be involved in skin homing. During the acute phase CD4+ cells express perforin.

3.6.3.4 Acute Generalized Exanthematous Pustulosis

Activated drug-specific CD4+ and CD8+ T cells producing the neutrophil-attracting chemokine CXCL8 (IL-8), infiltrate the skin of patients with acute generalized exanthematous pustulosis (AGEP) (Sect. 2.2.4.4) and can be detected in peripheral blood, in positive patch test biopsies, and in T cell lines and clones. CXCL8-producing effector memory T cells express mainly IFN-γ, GM-CSF, TNF, and sometimes IL-4 and IL-5. These cells express the chemokine CCR6 and aid infiltration and survival of neutrophils leading to the sterile pustular eruptions found in AGEP patients (Fig. 2.8).

3.6.3.5 Drug Reaction (Rash) with Eosinophilia and Systemic Symptoms

The pathophysiology of drug reaction (sometimes designated rash) with eosinophilia and systemic symptoms (DRESS), also called drug-induced hypersensitivity syndrome (DIHS), is still being worked out (see also Sect. 2.2.4.5). Activated CD4+ and CD8+ T cells expressing CCR10 and producing type 1 cytokines, chiefly IFN-γ, are found in the blood of DRESS patients in the acute phase and these cells increase in proportion to the severity of the skin reaction (Fig. 2.9). Interestingly, T cell clones from carbamazepine- and lamotrigine-sensitive patients react specifically with antigen-presenting cells apparently without the formation of reactive metabolites and processing, much like the situation with a superantigen. The T cell clones produce perforin and secrete IL-5 as well as IFN-γ, the former accounting for the eosinophilia associated with the syndrome. Many investigators believe that a concomitant human herpes virus 6 (HHV-6) reactivation with hypogammaglobulinemia caused by the drug is associated with the hypersensitivity syndrome. This remains to be established.

While mentioning DRESS, it is opportune to comment on drug-induced allergic hepatitis. As in DRESS, this condition is associated with fever, rash, eosinophilia, and liver infiltrates and the reaction is generally a type IV hypersensitivity response involving CD4+ cells, CD8+ cytotoxic lymphocytes, NK, Kupffer and dendritic cells. Type II hypersensitivities may also sometimes occur. There are two main hypotheses for the mechanism of drug-induced liver injury (DILI) caused by immune processes. First, the drug or active metabolite(s) acts as a hapten and binds to endogenous proteins forming conjugates that induce antibody- and/or T cell-mediated injury. Proponents of the second hypothesis suggest that most individuals are tolerant to immune-mediated DILI and reactions occur only when tolerance is

overcome. Although the cellular events remain poorly defined, knowledge of underlying mechanisms of idiosyncratic DILI is even more fragmentary.

3.6.3.6 Fixed Drug Eruption

Mediated by activated CD8+ T cells, fixed drug eruption (FDE) is a disease instigated by drugs in more than 95 % of cases. In regression, large numbers of CD8+ effector memory T cells of phenotype CD3+, CD45RA+, CD11b+, and CD27− are found in lesions in the epidermis. Reexposure to the culprit drug rapidly leads to a conversion of this benign state to one of aggressive cell damage. T cells secrete IFN-γ in high amount as well as TNF, perforin, granzyme B, and Fas ligand (FasL) which initiates cell killing by binding to its receptor FasR on keratinocytes. The presence of the "dormant" CD8+ T cells in "resting" lesions explains why patch testing is negative on normal skin but reactivation occurs when patches are applied to areas of residual lesions. For a clinical description of FDE see Sect. 2.2.4.6 and Figs 2.10 and 2.11.

3.6.3.7 Toxic Epidermal Necrolysis and Stevens–Johnson Syndrome

These diseases (Sect. 2.2.4.8) are provoked by drugs in more than 90 % of cases with sulfonamides, anticonvulsants, some NSAIDs, and allopurinol most frequently involved. It is not yet understood why and how a cutaneous adverse drug reaction very occasionally progresses to the life-threatening TEN or SJS. Clinical features of both syndromes are similar with the extent of necrotic epidermis/skin detachment greater in TEN (>30 %; Fig. 2.14) than in SJS (<10 %) and the predominance of lesions around mucosal orifices in SJS (Fig. 2.15). In fact, the two disorders are considered by many to be variants of the same disease with different severity. In TEN, blister fluid contains many activated HLA class I-restricted, drug-specific CD8+ CD56+ cytotoxic T cells with natural killer (NK) cell features. These kill lymphocytes and particularly keratinocytes via, according to different researchers, several mechanisms including the Fas/FasL (CD95/CD95L), TNF, granzyme B, perforin, TWEAK (TNF-like weak inducer of apoptosis), and TRAIL (TNF-related apoptosis-inducing ligand) pathways. These cytotoxic mediators are found in the serum as well as in blister fluid where levels are high and where they occur with other cytokines including IFN-γ, IL-10, and IL-18. Several studies suggest that TNF has an important role in TEN and this appears to be supported by the success of the TNF-targeted monoclonal antibody infliximab (see chapter 11, Sect. 11.1.3.3) in promoting the resolution of lesions in a number of patients.

These outlined findings are the conclusions assembled from a number of different investigators, but the explanations leave significant doubts since some key points remain unexplained. In particular, the number of infiltrating inflammatory cells in the skin lesions is claimed to be too few to explain the widespread killing of keratinocytes. In the first place, both of the two favored pathways to cell death, viz., granzyme B- and perforin-mediated exocytosis and Fas-FasL killing, are not restricted to TEN and SJS—both pathways are upregulated in some other adverse cutaneous reactions such as maculopapular erythema where widespread cell destruction does not occur. The second inadequacy of the dual pathway explanation is the need for cell-to-cell contact for killing when there seems to be not enough inflammatory cells for this to occur. These doubts have been expressed by Chung and coworkers in Taiwan whose investigations recently provided a better understanding of the immune mechanisms and biomarkers of TEN and SJS and promise new approaches for the management of these diseases. Gene expression profiling, PCR, and immunohistochemical methods identified granulysin rather than Fas, FasL, soluble FasL, granzyme B, or perforin as the major cytotoxic molecule responsible for keratinocyte necrosis in TEN/SJS. Granulysin, a member of the saposin-like family of membrane-disrupting proteins, is a cationic cytolytic and pro-inflammatory protein contained in the cytolytic granules of cytotoxic T lymphocytes and NK cells. Chung et al. showed that granulysin from blister fluid, in the 15 kD secretory form (a precursor of the 9 kD form), was present in a concentration two to four times higher that soluble

FasL, granzyme B, and perforin. Depletion of granulysin reduced cytotoxicity and when it was injected into mouse skin it produced TEN- and SJS-like skin necrosis. In addition to its cytotoxic effects, granulysin is a chemoattractant for other inflammatory cells and aids the expression of some chemokines and cytokines including RANTES (CCL5), MIP-1α (macrophage inflammatory protein-1α, CCL3), MCP-1 (monocyte chemotactic protein-1, CCL2), MCP-3 (monocyte chemotactic protein-3, CCL7), IL-1, IL-6, IL-10, and IFN-α.

In summary, the demonstrations of a pathogenetic mechanism for TEN/SJS and that secretory granulysin is a key toxic molecule responsible for disseminated keratinocyte killing open the way for the development of new diagnostic and therapeutic targets for the diseases. However, important questions concerning operative mechanisms in TEN and SJS remain. For example, what are the precise molecular mechanisms involved in the interactions between the offending drugs, HLA, and the T cell receptor? What are the precise steps between taking the drug and the secretion of granulysin? How is secretion of the cytolytic protein regulated? What are the identities of the determinants recognized in the immune processes? The beneficial effect of infliximab when used for selective TNF blockade in some cases of TEN, and the importance of TNF in causing direct cytotoxicity and apoptosis, must also be considered and somehow incorporated into a satisfying explanation of the pathogenesis of this intriguing toxidermia.

3.6.3.8 Delayed Cutaneous Drug Hypersensitivity Reactions. Conclusions

In reviewing what is currently known about the pathophysiology and mechanisms underlying the T cell-mediated delayed allergic drug reactions it is apparent that knowledge of the different cutaneous reactions is still widely incomplete and agreement, even on some basic processes, is often inconsistent or lacking. Absence of agreement on the identity of the often-bewildering number and nature of cytokines and chemokines said to be involved is particularly apparent for some of the drug-induced reactions. For the practicing clinician, especially those without specialty knowledge of immunology and dermatology, the field of drug-provoked cutaneous reactions is an area of great difficulty starting with the requirement of identifying the culprit drug, often amongst multiple drugs being taken. There then remains the need to undertake or order appropriate tests without further aggravating the condition; institute appropriate management measures; identify other drugs that may be a risk; and to take measures, including instruction of the patient, to avoid further reactions. A fairly recent interesting area of investigation that is particularly promising has emerged from demonstrated associations between HLA alleles, certain drugs, diseases such as TEN/SJS, and different human populations. Apart from the presentation of drug or drug metabolite to T cells, HLA alleles may also be responsible for genetic susceptibilities for drug-induced cutaneous reactions. As pointed out by Chung et al., "Understanding the molecular mechanism of the interaction of HLA, offending drugs and TCR, as well as CTLs/NK cells activation, would facilitate the development of new approaches for the management of SJS/TEN." With relevance to pathomechanisms and regard to classification of reactions, attention has been drawn to the particular cell type(s) recruited during the so-called second step of drug-induced skin inflammation following the involvement of drug-specific T cells in the first step. The important involvements of eosinophils with DRESS and neutrophils with maculopapular exanthema and AGEP illustrate the point.

As discussed in Chap. 4, the demonstration or detection of individual or patterns of cytokines and chemokines is a promising approach for improving the reliability and specificity of diagnosing some drug-induced cutaneous hypersensitivity reactions. Surprisingly, this diagnostic strategy still seems to be underutilized but significant advancements probably depend on first reliably implicating a suitable disease-specific marker or spectrum of markers. Finally, the allergenic determinants recognized in the cellular immune processes remain largely unexplored and undefined. Identification of the structures of drug–peptide complexes presented by the MHC and fine-structural detail of drug determinants

recognized by the T cell receptor remain areas sorely in need of both investigation and secure findings. Progress on these points is needed to reliably identify potentially cross-reacting drugs for patients and offers the possibility of selecting or tailor-making interfering inhibitory or competing molecules to mitigate drug-specific reactions.

3.7 Type II Hypersensitivity Drug Reactions

The classical drug-induced type II hypersensitivity is the well-known immune cytotoxic reaction to high doses of penicillin that results from binding of the drug to red cells. This causes the red cells to be recognized as foreign, resulting in IgM and IgG antibodies reacting with the drug–cell membrane protein complex. The antibody–antigen complexes so formed activate the classical complement pathway causing cell lysis and death and the antibody-coated red cells can interact with macrophages leading to Fc-mediated cell destruction by the reticuloendothelial system. Another example of a type II cytotoxic antibody-mediated drug reaction when the drug appears to form an antigenic complex with the red cell surface is drug-induced immune hemolytic anemia (DIIHA). The drugs most frequently associated with DIIHA are some cephalosporins (especially cefotetan and ceftriaxone) and penicillins (especially piperacillin). DIIHA can also be associated with red cell autoantibodies induced by the drug affecting the immune system without becoming bound to the red cell surface, that is, the drug does not participate in the antigen–antibody reaction. Such antibodies are referred to as drug-independent. Prototype drugs involved in drug-independent autoantibody formation are methyldopa and the chemotherapy drug, fludarabine. In this form of DIIHA the clinical and laboratory findings are identical to autoimmune hemolytic anemia. It is not known why drugs sometimes induce drug-independent autoantibodies to red cells or what mechanism is involved. The mechanism of DIIHA when the drug participates as the antigen is thought to proceed by attachment of the drug to the red cell in vivo, interaction with drug-reactive antibodies (usually IgG but may be IgM), and subsequent Fc-mediated cell destruction and clearance by macrophages. Activation of complement may occur leading to intravascular lysis and renal failure. Understanding the mechanisms involved in the action of drug-dependent antibodies is complicated by the observation that drugs that cause some of the worst reactions including hemolysis, renal failure, disseminated intravascular coagulation, and death appear to proceed via a different mechanism, often involving complement-activating antibodies. The so-called "unifying hypothesis" has been advanced to explain all three types of antibodies implicated in DIIHA. This hypothesis is based on known findings dating back to Landsteiner of the generation of three populations of antibodies to an injected hapten–protein complex—one population to the hapten, one to hapten plus carrier determinants, and one to the protein carrier. A further, and more recent, proposed mechanism for DIIHA is based on non-immune adsorption of the drug onto the red cell membrane. Cefotetan, often used prophylactically in some surgical procedures, is the most common cause of DIIHA with another cephalosporin, ceftriaxone, the second most common cause. For the period 1985–1997, the FDA reported 85 cases of cefotetan-induced hemolytic anemia with 15 (18 %) fatalities. It is therefore usually recommended that all cephalosporins should be avoided in patients where DIIHA is thought to be a possibility but in vitro hapten inhibition experiments have shown that only cefotetan and cephalothin inhibited anti-cefotetan IgG and IgM antibodies. It would be prudent to remember, however, that these were in vitro findings and the in vivo consequences may be quite different especially if allergic reactions (and IgE antibodies) are involved. With piperacillin, the third most common drug causing DIIHA, immune complexes seem to be involved.

As well as erythrocytes, other cells including platelets (thrombocytes) and some hematopoietic precursor cells can be affected by drug-induced type II hypersensitivity reactions. Drug-induced thrombocytopenia for example is increasing as more drugs are released and used. A number of different mechanisms appear to be involved. Drugs may bind covalently to the platelet mem-

brane producing a hapten–glycoprotein conjugate with an antigenic determinant(s) that is recognized by antibody. Drugs implicated in this form of thrombocytopenia include penicillins and cephalosporins in particular. Quinine, quinidine, sulfonamides, and NSAIDs may interact non-covalently with platelet membrane glycoproteins, including the von Willebrand factor receptor GPIb-IX-V (GP for glycoprotein) and activated integrins GPIIb/IIIa, forming drug–glycoprotein non-covalently linked complexes. For antibody binding to occur, the presence of the drug is essential—in the absence of the drug, antibodies do not bind to the platelet surface and thrombocytopenia does not occur. It remains uncertain whether the antibodies are directed to the drug alone or to the complex of drug and platelet glycoprotein. A third mechanism of drug-induced thrombocytopenia is seen with the antiplatelet GPIIb/IIIa inhibitor drugs lotrafiban, tirofiban, and eptifibatide, the novel cyclic heptapeptide from the venom of the southeastern pygmy rattlesnake. By binding to the glycoprotein complex, these drugs induce a conformational change and a new determinant to which antibodies bind and cause cell destruction. The drug does not physically form part of the determinant. Another inhibitor of platelet activation sometimes administered is abciximab, the Fab fragment of a chimeric human–mouse monoclonal antibody that binds to the platelet glycoprotein receptor GPIIb/IIIa. Some patients, even without prior exposure to the monoclonal agent, react to the mouse component of the hybrid, supporting the belief that natural antibodies may be involved in the recognition. Such recognition of murine antigens on a chimeric human–mouse antibody fragment is similar to the recognition by natural antibodies of the chimeric monoclonal antibody cetuximab (Sect. 3.1.1; Sect. 11.1.3.2). This humoral form of immune-mediated drug-induced thrombocytopenia is regarded as drug-specific since the antibodies are formed against the drug itself and platelets are destroyed in the process. In a fifth mechanism, drug induces the formation of autoantibodies to glycoproteins on the platelet surface. The antibodies bind to the platelet antigens without participation of the drug and the resultant thrombocytopenia can persist when the drug is withdrawn. The prototype drugs in this category are gold and procainamide. Finally, heparin and heparin-like drugs can induce thrombosis by binding to surface-bound soluble platelet factor 4 (PF4), a small chemokine CXCL4 that promotes coagulation and is released from the alpha granules of activated platelets during platelet aggregation. Antibodies to the heparin–PF4 complex bind to receptors on the platelet surface via their Fc pieces producing platelet activation. This mechanism is basically different from the other five described mechanisms in that activation and aggregation of platelets is the result rather than cell lysis and hemorrhage making the reaction more like a type III than a type II hypersensitivity response.

Acute agranulocytosis is rare but when it does occur, drugs are responsible in more than 70 % of cases. In its immune form, antibodies are produced to circulating neutrophils and/or myeloid precursor cells. Immune-mediated agranulocytosis is rapid in onset with symptoms generally occurring within a few days. Drugs commonly associated with the condition include quinine, quinidine, β-lactams, pyrazolones, propylthiouracil, clozapine, ticlopidine, carbamazepine, chlorpromazine, and some sulfonamides. Numerous other drugs have been implicated in one or only a few cases. Several mechanisms have been advanced although detailed and convincing evidence is not always offered. Some of the implicated drugs such as penicillins and aminopyrine are thought to act as haptens that elicit antibody formation against neutrophils and their subsequent destruction. In the case of aminopyrine-induced agranulocytosis, antibodies are directed to neutrophil cell membrane antigens modified by a reactive metabolite of the drug. Antibody recognition of metabolites was also demonstrated for metamizole and diclofenac in cases of agranulocytosis induced by these drugs. In addition to drug-dependent antibodies of the IgG and/or IgM class, autoantibodies were found in 13 cases of drug-related agranulocytosis due to penicillins, dimethylaminophenazone, propyphenazone, metamizole, and diclofenac. In the case of clozapine-induced agranulocytosis, the drug is converted to the reactive nitrenium ion which binds to cellular proteins and accelerates

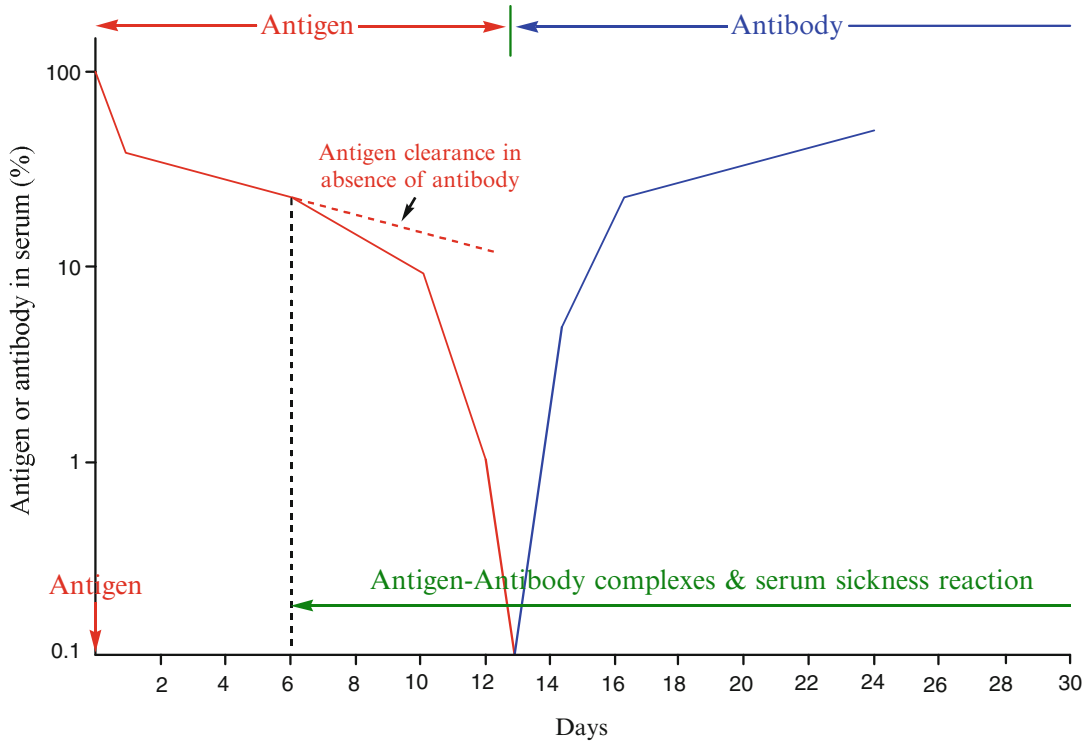


Fig. 3.18 Relationship between antigen introduction, subsequent immunological events, and time in serum sickness

apoptosis of neutrophils. Propylthiouracil was shown to lyse neutrophils via a complement-dependent mechanism. An immune mechanism does not seem to be involved with drugs such as ticlopidine, busulfan, chlorpromazine, and methamizole, each of which has a direct toxic effect on myeloid precursors.

3.8 Type III Hypersensitivity Drug Reactions

Serum sickness (see Sect. 2.2.3) can occur in response to foreign proteins such as streptokinase and to antitoxins, antivenins, and vaccines. As mentioned above in Sect. 3.2.8.2, type III drug-induced hypersensitivities, that is, antigen–antibody complex-mediated reactions, occur in some cases that closely resemble classical serum sickness. Penicillin has long been known to become antigenic by conjugating to proteins in vivo to produce drug–protein complexes that mediate type III hypersensitivity reactions. Thus, it can be said that penicillins can cause all four types of hypersensitivity reactions. Other drugs that produce similar serum sickness-like reactions include cephalosporins, sulfonamides, ciprofloxacin, tetracycline, lincomycin, NSAIDs, carbamazepine, allopurinol, thiouracil, propranolol, griseofulvin, metronidazole, furoxone, captopril, gold salts, methyldopa, halothane, fluoxetine, barbiturates, and monoclonal antibodies. β-Lactam drugs are considered the most common cause of serum sickness elicited by nonproteins but drugs by themselves are thought to be poor antigens for the production of the good antibody responses necessary to induce serum sickness. Circulating antigen–antibody complexes are formed after drugs become protein bound in vivo and stimulate IgG and/or IgM antibodies. The liberation of vasoactive amines is thought to play a part in tissue deposition. Antigen also interacts with complementary IgE antibodies on mast cells and basophils leading to the release of PAF and other mediators, platelet aggregation, and further

release of histamine and serotonin. The resulting increase in vascular permeability facilitates the deposition of immune complexes which, in turn, produces complement activation, the formation of C3a and C5a, an influx of inflammatory cells to the sites of immune complex deposition, and release of further inflammatory mediators. Drug immune complexes are normally rapidly cleared via the antibody Fc piece or complement binding to cells of the reticuloendothelial cells but if this does not occur, for example, because of the high concentrations of immune complexes, deposition of complexes in glomeruli, arteries, endocardium, spleen, and other organs and influx of inflammatory cells may result. In a graph that relates the time of occurrence of tissue lesions to the clearance of antigen and developing antibody production, Fig. 3.18 summarizes the immunologic events in the patient after antigen exposure. Serum concentration of protein-bound drug initially decreases sharply as a result of intravascular and extravascular equilibration and levels continue to decrease normally as the protein is catabolized until antibody levels increase, causing rapid immune elimination. The dashed red line in Fig. 3.18 at about day 6 represents the course of antigen decline in the absence of antibody-mediated antigen elimination. From about day 14, soluble circulating complexes of antigen with IgG or IgM form and may begin to deposit in a number of tissue sites leading to the clinical manifestations and pathologic findings of serum sickness.

Hypersensitivity vasculitis induced by drugs is another manifestation of a type III response. Drugs involved include some β-lactams, particularly, amoxicillin and cephalexin, cotrimoxazole, NSAIDs, monoclonal antibodies, and chemotherapeutic drugs such as tamoxifen and erlotinib. A proportion of small-vessel vasculitis patients have anti-neutrophil cytoplasmic antibodies. Although there is evidence of a pathogenic role for these antibodies and they are used as a diagnostic marker, operative mechanisms underlying this hypersensitivity state are still far from established.

Hypersensitivity reactions are one of a number of different mechanisms producing drug-induced lung disease. These reactions result from interaction of drug with the immune system and involve drug-specific antibodies or, more usually, drug-specific T cells. Eosinophilic pneumonia can be caused by almost any medication while reports of drug-induced hypersensitivity pneumonitis, a combined type III and IV reaction in a Th1/Th17 response, are increasing, particularly to antineoplastic drugs.

Summary

- For many drugs it has not been possible to explain allergic reactions on the basis of their chemical reactivity, protein-binding capacity, their biotransformed or degradative products, or the presence of a reactive impurity.
- Some allergic responses, sometimes even life-threatening as with anaphylaxis, occur on first exposure to a drug.
- There is at least one group of drugs, the neuromuscular blockers (and probably more to be identified), that can specifically elicit antibody-induced mast cell activation and release without first undergoing coupling to a macromolecular carrier. For these drugs, the di- or multi-valency which is an inherent part of the molecular structure, initiates mediator release by cross-linking cell-bound antibodies.
- The initial event in the activation of mast cells for mediator release is the binding of IgE antibodies to the high-affinity (K_a 10^{-10} M) FcεRI IgE receptor abundantly expressed on the mast cell and basophil surfaces.
- Released preformed mediators of inflammation and anaphylaxis stored in the cytoplasmic granules of mast cells include histamine, heparin, platelet-activating factor (PAF), serotonin, the enzymes tryptase, chymase, and carboxypeptidase, and eosinophil, neutrophil, and monocyte chemotactic factors. Newly synthesized released mediators include prostaglandin D_2, thromboxanes, and leukotrienes LTB_4, LTC_4, and LTD_4. A host of cytokines (pro- and anti-inflammatory), chemokines, and chemotactic, stimulating, and growth factors are also released.
- A second receptor for IgE, the low-affinity receptor FcεRII also known as CD23, is

expressed on airways smooth muscle cells and several types of hematopoietic cells including mature B lymphocytes, macrophages, monocytes, dendritic cells, and eosinophils.

- Histamine is synthesized from L-histidine by the inducible enzyme L-histidine decarboxylase and inactivated by histamine *N*-methyltransferase-catalyzed methylation of the imidazole ring and oxidative deamination of the primary amino group catalyzed by diamine oxidase.
- The physiological and pharmacological effects of histamine are mediated through four different receptors H_1, H_2, H_3, and H_4, all members of the 7-transmembrane g protein-coupled receptor (GPCR) family with amino terminal glycosylation sites and phosphorylation sites for protein kinases A and C.
- Pathophysiological effects resulting from stimulation of the H_1 receptor include those responses seen in immediate allergic reactions, viz, redness, itch, swelling, asthma, anaphylaxis, bronchoconstriction, and vascular permeability.
- H_2 receptors appear to mainly mediate suppressive activities of histamine including gastric acid secretion, heart contraction, cell proliferation, differentiation, and some effects on the immune response.
- The H_3 receptor regulates the synthesis and release of histamine and also has a regulatory role in the release of neurotransmitters such as serotonin, dopamine, and norepinephrine.
- The H_4 receptor is functionally expressed on mast cells, eosinophils, monocytes, dendritic cells, and CD8+ T cells. The receptor exerts a chemotactic effect on several cell types associated with immune and inflammatory responses such as allergy, asthma, rheumatoid arthritis, and inflammatory bowel disease.
- LTC_4 and LTD_4 are powerful mediators of asthma, airway hypersensitivity, and allergies inducing bronchoconstriction, increasing vascular permeability, and promoting mucous secretion. LTE_4 is present in greatest amount in vivo where it induces bronchial eosinophilia and airway hyperresponsiveness. The bronchoconstriction provoked by LTE_4 is strong in patients with aspirin-sensitive asthma but much weaker in other asthmatics. LTD_4 is much more pronounced in asthmatic patients not sensitive to aspirin.
- Cysteinyl leukotrienes are generated de novo from arachidonic acid by phospholipase A2 with the initial participation of 5-lipoxygenase-activating protein and the enzyme 5-lipoxygenase.
- The two human cysteinyl leukotriene receptors $CysLT_1R$ and $CysLT_2R$ do not bind the three cysteinyl leukotriene ligands equally: for $CysLT_1R$, $LTD_4 > LTC_4 = LTE_4$; for $CysLT_2R$, $LTC_4 = LTD_4 > LTE_4$.
- PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a phospholipid of relatively simple but unique structure, produces both the signs and symptoms of anaphylaxis. PAF is also an important mediator in asthma and septic shock. Recent findings in the mouse identified a second pathway of anaphylaxis involving the IgG receptor FcγRIII and the release of PAF as the major mediator.
- Recruitment of the Syk kinase and subsequent phosphorylation activation steps involving Lyn lead to mast cell activation demonstrating the importance of protein tyrosine kinases in the pathways that result in allergic inflammation and anaphylaxis.
- Sphingosine-1-phosphate, a major regulator of the vascular system and B and T cell trafficking, is elevated in the lungs of asthmatics where it regulates pulmonary epithelium permeability and is thought to contribute to the pathogenesis of anaphylaxis and rheumatoid arthritis.
- Mechanisms of anaphylaxis independent of IgE and including PAF-induced shock have been suggested.
- Urticaria is a heterogeneous disease with many subtypes caused by a range of agents and stimuli. Subtypes include urticaria due to genetic or immune mechanisms, urticaria with an autoimmune basis and nonimmune-mediated urticaria, and angioedema.
- The combination of actions of ACE inhibitors of decreasing angiotensin II and aldosterone and increasing and maintaining bradykinin levels may lead to fluid extravasation into subcutaneous tissue ultimately producing angioedema.

- Angioedema may also occur following administration of angiotensin II receptor-binding inhibitors such as losartan.
- The allergen-induced late phase reaction has features of a cell-mediated hypersensitivity response but shows some significant differences best illustrated by the different cytokine profiles.
- An early clue to specific immune recognition of "small," unbound chemicals and hence drugs was the demonstration of selective interaction of nickel ions with an MHC-II-bound peptide.
- Abacavir–HLA binding studies indicate that the drug changes the shape of the antigen-binding cleft. This results in preferred binding of smaller amino acids, an alteration in the repertoire of self-peptides that bind HLA-B*57:01, and a T cell response to self-proteins presented only in the presence of abacavir.
- Carbamazepine, a drug strongly associated with HLA-B*15:02, binds to this allotype and alters the repertoire of presented self-peptides. The most likely binding site on the carbamazepine molecule is the ketone of its 5-carboxamide group on the tricyclic ring.
- The mast cell and possibly the basophil appear to be the cells most likely involved in the desensitization of patients to drug allergies.
- Drug-induced delayed-type cutaneous hypersensitivity reactions manifest mainly as exanthemas, mediated by CD4+ and CD8+ CD3+ T cells in the dermis and epidermis. There are two phases of the hypersensitivity response, sensitization (or initiation or induction) involving keratinocytes, Langerhans', and dendritic cells and elicitation via T cells.
- Some progress has been made in identifying mechanisms underlying the different drug-related skin eruptions with an immunological pathogenesis but more precise definitions are needed. Individual important drug-induced delayed reactions include allergic contact dermatitis, psoriasis, maculopapular exanthema, AGEP, DRESS, FDE, TEN, and SJS.
- Drug-induced allergic hepatitis, as in DRESS, is associated with fever, rash, and eosinophilia. The reaction is generally a type IV hypersensitivity response involving CD4+ cells, CD8+ cytotoxic lymphocytes and NK, Kupffer, and dendritic cells. Type II hypersensitivities may also sometimes occur. Knowledge of mechanisms underlying idiosyncratic drug-induced liver injury is limited.
- Granulysin appears to be a key toxic molecule responsible for disseminated keratinocyte killing in TEN/SJS.
- Examples of type II cytotoxic antibody-mediated drug reactions include drug-induced immune hemolytic anemia, drug-induced thrombocytopenia where a number of different mechanisms are involved and acute agranulocytosis in which more than 70 % of cases are caused by drugs
- Type III drug-induced hypersensitivities, that is, antigen–antibody complex-mediated reactions, occur in some cases that closely resemble classical serum sickness. Drugs implicated include β-lactams, sulfonamides, ciprofloxacin, tetracycline, lincomycin, NSAIDs, carbamazepine, allopurinol, thiouracil, propranolol, griseofulvin, metronidazole, furoxone, captopril, gold salts, methyldopa, halothane, fluoxetine, barbiturates, and monoclonal antibodies. Circulating antigen–antibody complexes are formed after drugs become protein bound in vivo and stimulate IgG and/or IgM antibodies. The liberation of vasoactive amines is thought to play a part in tissue deposition.
- Hypersensitivity vasculitis induced by drugs is another manifestation of a type III response. Drugs involved include some β-lactams, particularly, amoxicillin and cephalexin, cotrimoxazole, monoclonal antibodies, and NSAIDs.
- Hypersensitivity reactions are one of a number of different mechanisms producing drug-induced lung disease. Eosinophilic pneumonia can be caused by almost any medication while reports of drug-induced hypersensitivity pneumonitis, a combined type III and IV reaction in a Th1/Th17 response, are increasing, particularly to antineoplastic drugs.

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Diagnosis of Allergic Reactions to Drugs

4

Abstract

In diagnosing drug allergies, history, skin testing, some in vitro laboratory tests and the challenge test are the backbone of the investigation. If skin prick testing elicits no reaction, intradermal testing is usually employed. The latter test is more sensitive but produces more false positives. The COADEX classification should be used to assess clinical relevance of positive patch tests. Assays for drug-specific serum IgE antibodies are useful in cases of skin test-negative or equivocal reactors or when skin tests are unreliable/unavailable. In interpreting results of IgE antibody tests, receiver-operating characteristic (ROC) curves provide more information to aid discrimination between positive and negative results. Drug challenge is the best way to confirm an allergic reaction, and it is considered to be the "gold standard" in the diagnosis of drug hypersensitivities. In anaphylaxis, the ratio of total to mature tryptase is typically less than 10. Given the technical improvements made with BAT and the test's validation for a number of drugs, it continues to be applied to many drug reactions. Nonproliferation-based in vitro assays of cell surface activation markers, cytokines, chemokines, and skin-homing receptors will be increasingly applied to diagnosis. ELISPOT assays (e.g., for IFN-γ and granzyme B) show potential for diagnosis and the chemokine CCL27 and CLA are promising markers for aiding efforts to understand the relationship between T cells, drugs, and adverse delayed skin reactions.

In diagnosing drug allergies, the patient's history, skin testing, some in vitro laboratory tests and the challenge test are the backbone of the investigative procedures. A successful diagnosis of drug allergy can be particularly difficult since each of these investigations has limitations and drawbacks. The history is usually pieced together sometimes from inadequate descriptions and recall by patients taking different drugs simultaneously; agents used for skin testing are not always ideal and are usually unstandardized; suitable laboratory tests are not always available and sensitive enough for testing reactions with a humoral or cell-mediated immune basis; and drug

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_4, © Springer Science+Business Media, LLC 2013

challenge tests are involved, possibly harmful for the patient and not always sensitive enough. Even so, by application of more than one of these four basic investigations and sometimes supplementing the diagnostic process with some more specialized tests, an accurate diagnosis can usually be achieved. The four basic diagnostic investigations will be reviewed together with other tests some of which are essentially still research tools with yet to be established clinical diagnostic reliability. Some of these more "specific" tests (that is "specific" in terms of the restricted or specified nature of what they measure rather than the absolute preciseness of the measurement) may eventually occupy a regular place in the evaluation and management of patients with drug allergies.

4.1 Case History

In assessing a case of drug allergy, the patient's clinical history is the most important component of the diagnostic process. It should be self-evident that diagnosis is not just the selection, ordering, and subsequent assessment of tests some of which might not be needed if the physician spends the necessary time on a little forensic questioning and analysis needed to assemble an adequate case history. As part of medical training and from the experience of practice, clinicians in all disciplines of medicine learn the importance of the medical history, or anamnesis, of a patient. The symptoms reported by the patient together with the clinical signs ascertained by direct physical examination, sometimes confirmed by clinical and/or laboratory tests, enables the clinician to make a diagnosis which is essentially based on pattern recognition, context, and probability. In addition to the obligatory and standard patient information that needs to be gathered such as age, weight, height, past medical history, family history, home environment, work, diet, medication, allergies, habits, smoker or not, alcohol consumption, and so on, assessment of a case of possible drug allergy must include a series of relevant and specific drug-related questions. Obtaining the following information is aimed at providing answers that will help the diagnosis, the selection of measures for immediate treatment if needed, and the formulation of a future avoidance strategy.

1. A list of all the medications the patient is, or has been, taking including over-the-counter preparations.
2. How much was (is being or has been) taken and for how long?
3. Which drug is the prime suspect of causing the reaction and why?
4. When did the reaction occur and how long did it last?
5. What was the temporal sequence of events between the initiation of therapy and the onset of symptoms?
6. Did the reaction occur on first exposure to a drug?
7. What were the manifestations of the reaction? For example, if there was a skin reaction, describe it. Did any swelling, choking, shortness of breath, or itching result? Get a list of *all* symptoms.
8. Has the patient recently been subjected to any medical or dental procedures such as major or minor surgery, radiographic investigation, immunization, or tooth filling or extraction?
9. Has the patient ever had a previous reaction to the suspected drug or to any other drug and is there a history of drug allergy?
10. Is the patient atopic and is there a family history of drug allergy or allergy in general?
11. Does the patient have a viral infection or has he/she had one recently?
12. Does the patient have any other disease, in particular, asthma, cystic fibrosis, diabetes, etc.?
13. Questions on home environment, pets, hobbies etc.

Answers to questions 3, 4, 5, and 7 can go a long way toward helping to establish a firm diagnosis. In relation to points 4 and 5, information on the temporal sequence of events can provide essential information needed to help determine the mechanism of the reaction. Immediate, IgE-antibody-mediated reactions that can range from a simple rash to full-blown anaphylaxis generally occur from only a few minutes to 1 h after drug administration. Delayed or late reactions may occur from more than 1 h up to several days after

administration. These reactions that may present as maculopapular rashes, fixed drug eruptions, and different exanthems suggest a drug-specific T cell-mediated mechanism. In response to question 7, a good description, or better, a direct view or photograph (e.g., of skin reactions) of the clinical manifestations of the reaction, can be very informative. Symptoms that result from the activation of mast cells such as anaphylaxis, bronchospasm, angioedema, and urticaria indicate an immune mechanism mediated by drug-reactive IgE antibodies. Note, however, that some drugs, for example, vancomycin and contrast media can have a direct, nonimmune effect on mast cells. Cutaneous reactions of the type mentioned above generally indicate responses mediated by T cells.

Some patients are taking multiple drugs so it is often difficult to identify a culprit drug. This is a particular problem in surgery, when many drugs are often administered in a short time. Reactions that occur during anesthesia usually cannot be determined reliably without detailed investigations. Difficulties in identifying the culprit drug and the sequence of events before and after the reaction may also occur in patients who are infants or young children, aphonic, dyspneic, or unconscious, where skin pigment masks some cutaneous reactions and in specific clinical situations, for example, during childbirth and hemodialysis. In all of these cases it is the physician or anesthetist who is in the position and has the responsibility of identifying the provoking agent and ensuring that this sometimes potentially vital information is recorded for future access. This may take the form, for example, of a letter given to the patient or the patient being advised to wear a warning chain or bracelet.

In conclusion, although taking a detailed drug history is an integral part of assembling a patient's medical history, in cases of suspected drug reactions it is a task that is all too often inadequately performed by busy clinicians recording incomplete information in a perfunctory way. It should be remembered that a possible consequence of failing to document and subsequently consult a history of a drug reaction(s) before prescribing medications could be the basis of a successful malpractice suit.

4.2 Skin Testing

4.2.1 General Aspects of Skin Testing for Drug Hypersensitivity

The amount of information gained from a patient's history that is potentially useful in a diagnostic investigation of a suspected drug allergy will vary with different patients. After gathering and recording the most comprehensive and detailed history that is obtainable from a patient, the usual next step in the diagnostic process is to make a clinical assessment and decide whether the available information suggests that a hypersensitivity reaction is a possibility or if the probability of such a reaction is so low that an allergic reaction is considered to be most unlikely. In the latter case, a drug challenge test might be carried out to eliminate any suspicion of an allergic drug sensitivity while in the former case some testing to investigate the possible presence of a hypersensitivity should be pursued. The first test to consider in this situation, and usually the first choice, is skin testing. Skin tests, in prick or intradermal form, are an appropriate diagnostic tool for hypersensitivity reactions of the immediate type, for example, in cases of suspected anaphylaxis, angioedema, bronchospasm, urticaria, conjunctivitis, and allergic rhinitis. The risk of systemic reactions is lower with skin prick and patch testing than with intradermal testing. Patients with a history of previous anaphylactic reactions or uncontrolled asthma, pregnant women, and small children should be considered at higher risk of systemic and anaphylactic reactions but the risk of fatality due to prick and patch tests is remote and anaphylactic reactions are rare. The patch test and, sometimes after very careful consideration of the risks, late readings of the intradermal test are appropriate for investigating drug reactions such as contact dermatitis, erythema multiforme, exanthematous drug eruptions, fixed drug eruptions, leucocytoclastic vasculitis, Stevens–Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN). Note that for

these latter high-risk patients, a careful risk to benefit analysis must be undertaken and, if it is decided it is in the patient's best interest to proceed with testing, test solutions should start at very high dilutions and all safety precautions, including hospitalisation of the patient (see Sects. 4.2.2 and 4.4), should be taken. In cases of severe late and delayed reactions, the time intervals between tests may be extended or patch testing alone may be employed.

Many of the drugs that will need to be tested are available only in tablet or capsule form. In these cases, the tablet, pill, or capsule contents should be accurately weighed before grinding to a fine powder in a mortar. Sugar or other protective coating on pills should be removed first. Since most solid dosage forms are formulated with other substances, and bearing in mind the specified dose per tablet/capsule of drug, the quantity of the powdered material sufficient to contain the desired amount of drug needed to prepare the skin test solution or patch test is obtained by weighing out the appropriate portion of the original weight of the tablet/capsule. A prick test solution is prepared by dissolving or dispersing the desired concentration of powdered material in sterile physiological saline or a 1:1 solution of glycerine:saline. For some drugs, adjustment of the pH or use of other solvents may be needed to aid solubility. This was done, for example, to increase the solubility of trimethoprim and sulfamethoxazole where 0.1 M sodium hydroxide and benzyl alcohol were added (see Sects. 6.2.1.2.1 and 6.2.2.2). Lack of water solubility may also be overcome by dissolving the drug in dimethyl sulfoxide (DMSO) and further diluting with sterile physiological saline to the desired concentration. In all such cases of where special diluents are used, the diluent itself must be used as a control.

Prior to proceeding with skin test studies on any drug, the optimal test concentration for that drug must be determined. This is the highest concentration that produces no skin reactions in a group of control subjects who have never been exposed to the drug, and in a group of nonallergic patients who have been exposed to the drug, but which will elicit a positive response in patients allergic to the drug.

Before undergoing skin testing, the patient should discontinue taking antihistamines at least 5 days before testing commences, and if the patient is pregnant the testing physician should be informed. Other drugs that must be discontinued prior to skin testing include β-adrenergic blocking agents, corticosteroids, including preparations for topical application, tricyclic antidepressants like amitryptyline and histamine H_2-receptor antagonists. There appears to be no universal agreement that the latter two groups of drugs interfere. A consent form setting out the reasons for the test and its procedures, benefits, and risks should be read and signed. Once these necessary preparations have been completed, the patient's blood pressure, pulse, peak expiratory flow, and oxygen saturation levels are measured and recorded. These measurements may be repeated during and at the conclusion of the test.

4.2.2 Skin Prick Test Method

Skin prick testing is performed on the volar aspect of the forearm or on the back. A solution of the drug is placed on the skin and a new lancet or fine-gauge needle is passed through the drop pricking the top layer of skin without drawing blood. This allows the test solution to gain access to cells of the dermis. The excess solution remaining on the skin is wiped away. If a number of solutions are to be tested, a testing grid can be placed or drawn on the skin and the drops are placed in the center of each grid square. As a positive control, histamine 10 mg/ml (1 % w/v) or codeine phosphate 90 mg/ml (9 % w/v) is included while the vehicle used for the test solutions (often physiological saline or glycerin-saline) is used as a negative control. Results are read and recorded after 15–20 min in the case of immediate reactions and after 24–72 h (and sometimes longer) for late and delayed reactions. A longer time interval before reading is often necessary for aminoglycoside antibiotics like neomycin (see Sect. 6.1.5.1). The size of the wheal and erythema (flare) reaction can be recorded in different ways and different scoring systems are employed. For documenting the

reaction, the outer margins of the wheal and flare can be traced on the skin using ink that is transferable to adhesive translucent cellophane tape. Alternatively, the reactions can be traced directly onto translucent tape placed over the reaction site. The recorded area of the reaction on the tape can be quantified by weighing, planimetry, or by computerized scanning. Calculation of the so-called mean diameter is one of the most frequently used procedures to compare and record skin test reactions. This involves the measurement of the largest diameter (D) of the wheal and a diameter perpendicular to this (d). The mean diameter is then obtained from $(D+d)/2$. For inhalant and many other allergens, a 3 mm or greater wheal mean diameter in the prick test is generally considered a positive reaction. The number of drugs employed in skin testing, although growing, is still too few to confidently assign an overall figure for positivity (e.g., wheals resulting from most anesthetic agents are usually smaller than those resulting from other agents causing anaphylaxis), and because penicillins are the drugs most studied in skin testing, criteria for positivity with these drugs tend to be applied when reading the results of skin tests with other drugs. In practice, this means that a wheal diameter at least 3 mm greater than the negative control is considered a positive result in most tests on drugs. For some drugs, a wheal diameter at least half the diameter resulting from the positive control is taken as a positive reaction by some investigators (see for example Sect. 7.4.3.3.1). Reactions read at later time intervals to detect late-phase reactions and delayed reactions involve the documentation of induration in particular but also any erythema, papulation, and vesicles. Any erythema with infiltration is considered to be a positive reaction. Late-phase reactions are IgE antibody-dependent but differ from immediate type I reactions by the involvement of neutrophils, eosinophils, and mononuclear cells (see Sect. 3.3). Whether or not the investigation is aimed at detecting late or delayed reactions, skin prick test sites should also be read 1 day after the tests.

If skin prick testing elicits no reaction, intradermal testing is usually employed. The latter test is more sensitive than the prick test but produces more false positives, that is, it is less specific. For some practitioners of skin testing, the extra sensitivity provided by the intradermal test and the tendency of the prick test to produce more false negatives are more than compensated for by the greater specificity of the prick test. This superior specificity, it is argued, correlates better with clinical allergy and the sensitivity is said to be adequate when sufficiently potent extracts are used. Additional advantages of prick testing are claimed to be the presence of glycerin (usually at 50 % concentration) that is thought to provide better stability for extracts, superior patient comfort and safety, and an economy of time in the test's application.

4.2.3 Intradermal Testing

Intradermal tests need to be performed only when the prick test gives negative results with testing beginning after a 15–20 min break. Solutions for testing are prepared under a laminar flow hood in sterile physiological saline or sterile saline with 0.5 % phenol no longer than 2 h before administration. Intradermal testing is normally contraindicated in patients who have developed SJS, TEN, erythema multiforme, or leucocytoclastic vasculitis although testing such high-risk patients may be judged to be necessary in special circumstances and with all safety precautions in place (see Sects. 4.2.1 and 4.4). So far, there appears to be no report of skin testing provoking or causing the reoccurrence of toxic epidermal necrolysis.

Using a 25-gauge needle with the bevel uppermost at an angle of 15–20° to the skin surface, 0.02–0.05 ml of the test solution is injected intradermally (Fig. 4.1) on the forearm or back to produce a small blister or bleb (Fig. 4.2). Depending on the drug and the severity of the patient's drug reaction, the initial injection may range from a small dilution of 1:10 or 1:100 of the prick test concentration to more extreme dilutions of up to 1:100,000. If no reaction is seen, the concentration is increased in logarithmic steps until the final concentration is reached and this maximum

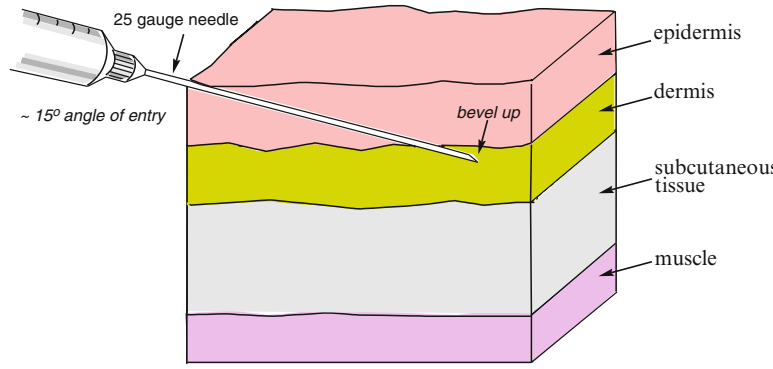

Fig. 4.1 Diagrammatic representation of an intradermal skin test

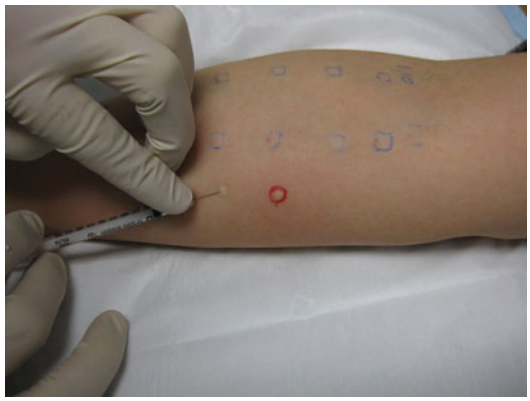

Fig. 4.2 An intradermal skin test being performed. Note the *small blister* or *bleb* formed from the solution injected into the dermis (Photograph courtesy of Dr. Paul A.J. Russo, Department of Clinical Immunology and Allergy, Royal Adelaide Hospital)

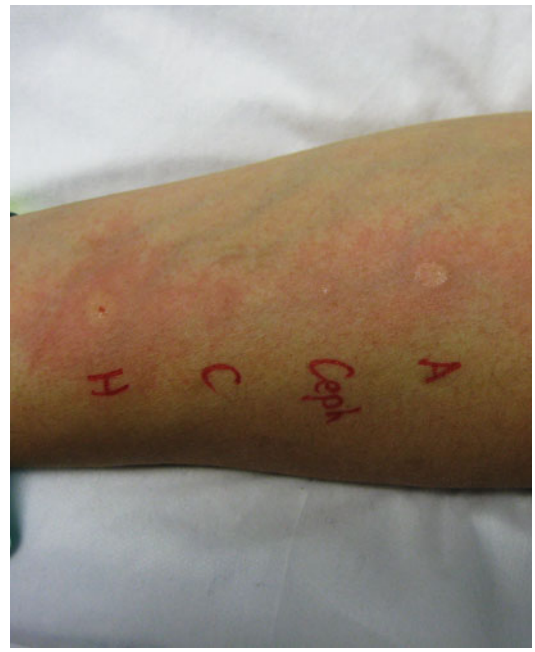

Fig. 4.3 Clear positive wheal and flare reactions to amoxicillin (A) and histamine (H) control solution (10 mg/ml) following intradermal testing (Photograph courtesy of Dr. Paul A.J. Russo, Department of Clinical Immunology and Allergy, Royal Adelaide Hospital)

concentration should not be exceeded. Histamine base 0.01-0.1 mg/ml (0.001-0.01% w/v) and the solvent for the drug are included as positive and negative controls respectively. Some regard a positive test as the appearance of an erythematous wheal after 20 min with a diameter at least twice that of the initial bleb. Probably a more rigorous and widely accepted positive threshold is an increase in diameter of more than 3 mm over the initial 20 µl injection bleb (usually ~ 2 mm) accompanied by erythema (Fig. 4.3). This threshold caters for different sizes of the bleb formed from the injected solution and generally means that a positive reaction has a diameter of more than 5 mm. Tests should be read after 15–20 min for immediate reactions and after 48 and 72 h for delayed reactions. With some drugs even later readings may be needed. The positive predictive value of a skin test is, in general, high so a positive result can be taken as diagnostic but a single negative result cannot necessarily rule out drug

allergy. After a careful analysis of the risks and benefits, consideration should be given to proceeding to a second test using the next step-up concentration of drug. If the intradermal test remains negative, the patient should be contacted 1 week later and asked whether or not the test site is still negative, or the patient should be instructed to return for the site to be inspected.

4.2.4 Patch Tests

4.2.4.1 General Aspects of Patch Testing

Adverse drug reactions affecting the skin are a frequently seen condition and, as for other manifestations of adverse drug reactions, for patients receiving multiple medications it is often difficult to identify the culprit drug from history alone. Patch testing with drugs, in both pure and commercial form, is valuable in helping to determine the cause of drug-induced cutaneous drug reactions and for studying the pathophysiological mechanisms underlying the reactions. A strong positive feature of the patch test is that it is both a screening test for hypersensitivity and a provocation test in the target organ, the skin, where it can be seen as reproducing the disease. Unlike patch testing that requires no hospital surveillance during testing, rarely provokes anything more than a mild reaction and can be used with commercial forms of drugs, intradermal tests with drugs carry a greater risk and can only be performed with pure dissolved material in free, sterile solution. On the other hand, patch tests are less sensitive than intradermal tests. The specificity and sensitivity of patch tests is said to be in the range 70–80 % depending on the test agent but in four separate studies where a single drug was implicated with high imputability, drug patch tests proved positive in 31.7–50 % of patients with a cutaneous adverse drug reaction. Results of patch tests depend on the clinical features of the cutaneous drug reaction, the drug tested and its concentration, the vehicle used, and sometimes on the skin test site. It has been claimed that the test is valuable for investigating generalized eczema, maculopapular rash, photosensitivity, baboon syndrome, contact dermatitis, fixed drug eruption, lichenoid rash, and acute generalized exanthematous pustulosis, and it may be of value for drug reaction (rash) with eosinophilia and systemic symptoms (DRESS). It is thought to be of less use in investigating urticaria, SJS and TEN. A significantly higher number of positive reactions appear to occur with drug-induced maculopapular rashes than with urticarial or erythrodermic reactions. Positive reactions are often observed with some drugs, for example, β-lactams particularly amoxicillin, cotrimoxazole, pristinamycin, hydroxyzine, pseudoephedrine, carbamazepine, heparinoids, diltiazem, diazepam, and tetrazepam. The skin site tested can also be important. In fixed drug eruptions, patch tests should be performed on both normal skin and on residual pigmented skin sites of the eruption. In the case of toxic necrolysis, a positive patch test with cotrimoxazole was obtained on skin previously affected by necrolysis but not at other less, or unaffected, sites.

4.2.4.2 Concentrations of Drug

An important advantage of patch tests is their capacity to utilize virtually any form of a commercialized drug. When used with a pure drug, a 10 % solution in water or alcohol or a 10 % dispersion in petrolatum is employed. Some drugs require a specific vehicle—for example, alcohol, not water, should be used for estrogen and progesterone, and β-lactams tend to give false negative results in an aqueous vehicle and should therefore be tested at 5–10 % in petrolatum. In some cases olive oil, rape oil, or acetone may be used. Test materials prepared from tablets and other formulations always contain additives (diluents, binders, pigments, sweeteners, lubricants, disintegrants, granulating agents), so a higher quantity, usually 30 %, of powdered tablet, pill, or capsule contents is mixed with the diluent for patch application (see Sect. 4.2.1). Whenever possible these excipient substances should also be tested. If pure drug is used for testing, concentrations should begin at about 0.1 % and progress to 1–10 % if results are negative. If DRESS, SJS, or TEN patients must be tested, with, for example, aciclovir, carbamazepine, or

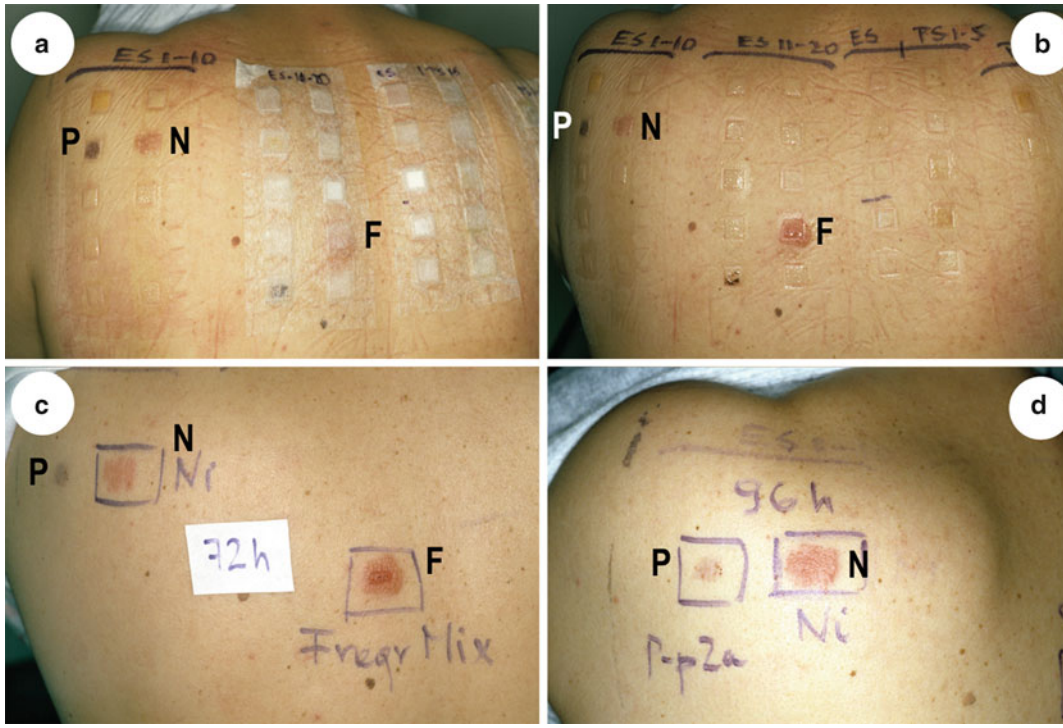

Fig. 4.4 Patch tests showing (**a**) patches in place during development of reactions and (**b**) immediately after their removal, 2 days after being applied. A strong positive reaction (++) to nickel (N) and an extreme positive reaction (+++) to fragrance mix (F) are visible in (**b**). An equivocal reaction (?+) to *p*-phenylenediamine (P) is seen in (**c**). After 4 days further development (**d**), both the nickel (+++) and *p*-phenylenediamine (+) reactions have intensified. For further details of notation of reactions see Fig. 4.5 and Table 4.1. From Spiewak R. Patch testing for contact allergy and allergic contact dermatitis. The Open Allergy Journal 2008;1:42. Reproduced with permission of the author

pseudoephedrine, testing should start with much lower concentrations to avoid any relapse of a cutaneous adverse drug reaction.

4.2.4.3 Method, Materials, and Reading

After obtaining informed consent from the patient, patch testing should begin 6 weeks to 6 months after healing of any cutaneous adverse drug reaction and 4 weeks after discontinuing immunomodulating drugs such as glucocorticoids or cyclosporin and topical corticosteroids. Due to hormonal effects on test results, patch testing should not be performed during pregnancy nor should it be done after a patient has experienced strong ultraviolet exposure, for example, after a seaside holiday. Otherwise, patients should be in good health, free from virus and other infections, fever, and inflammation. With the possibilities in mind of substituting another drug and gaining an understanding of cross-reactions, related drugs with similar pharmacological action and/or chemical structure should be tested along with the suspected culprit drug. Patch tests should be applied to the upper back usually at a distance of about 2–4 cm from the centerline using Finn Chambers on Scanpor® (a hypoallergenic tape) (Epitest Ltd Oy, Tuusula, Finland); Van der Bend Square Chambers (Brielle, The Netherlands); IQ Chambers™ (Chemotechnique, Vellinge, Sweden); or T.R.U.E. Test® (Thin-layer Rapid Use Epicutaneous Test, SmartPractice, Denmark ApS). Figure 4.4a shows patch tests in place on the back of a patient, clear positive reactions (++ and +++) to two contact allergens, nickel and a fragrance mix, after removal of the patches (b, c, d) and an equivocal positive reaction (?+) (c) and weak positive reaction (+) (d) to

Table 4.1 Scoring of patch test reactions

Score	Clinical picture	Interpretation
NT		
IR	Different types of reactions (e.g., vesicles, blister, necrosis)	Irritant reaction
–	No reaction	Negative reaction
? or ?+	Faint erythema only—no infiltration	Doubtful or equivocal reaction
+	Erythema, infiltration, possibly discrete papules	Weak positive reaction
++	Erythema, infiltration, papules, vesicles	Strong positive reaction
+++	Erythema, infiltration, confluent vesicles	Extreme positive reaction

Typical scoring and notation system used when reading patch test results. See for example, Wilkinson DS, et al. Acta Derm Venereol. 1970;50:287; Spiewak R. The Open Allergy J. 2008;1:42; Lachapelle J-M, Maibach HI. Patch testing and prick testing. A practical guide official publication of the ICDRG, 3rd edn. Berlin: Springer-Verlag;2012
Note that follicular reactions (usually denoted by F and not shown here) can be categorized as doubtful reactions
NT not tested

p-phenylenediamine. Some practitioners state that in order to avoid immediate reactions, patch test reactions should be read at 20 min while others believe that such reactions should be tested and ruled out before patches are applied. Reactions are read after 48 and 72 or 96 h. In some cases a reaction occurs in less than 2 days (e.g., abacavir) while with some other drugs such as corticosteroids, aminoglycoside antibiotics, and phenylephrine, reactions may occur after 6 or 7 days. If the result is negative on day 4, a further reading should be carried out on day 7. Reactions based on morphology are scored as shown in Table 4.1 with reactions rated as +, ++, or +++ interpreted as increasingly positive (Fig. 4.5). Positive reactions to structurally related compounds may reflect cross-reactions but "polysensitization" or reactions to a number (e.g., at least five or six) unrelated compounds may indicate a highly developed drug sensitivity or the so-called angry back or excited skin syndrome reflecting false positives. A complete absence of positive reactions despite a history highly suggestive of drug sensitivity may be a false negative response due to drug concentrations that are too low, insufficient occlusion, an inappropriate vehicle, reading at too early a time, absence of a drug metabolite, or reduced or impaired immunoreactivity of the patient. Any repeat test should be carried out after a delay of 2 months. Nonspecific irritant reactions can be induced by some drugs including colchicine, misoprostol, used for the prevention of nonsteroidal anti-inflammatory drug (NSAID)-induced gastric ulcers and by sodium lauryl sulfate which is included in some commercial drug formulations.

4.2.4.4 Clinical Relevance of a Positive Patch Test

A positive patch test (indicating contact allergy) is not necessarily a positive diagnosis of allergic contact dermatitis as indicated by the fact that some patients with a positive patch result never experience clinical symptoms after exposure to the test agent. The so-called COADEX classification is useful in the attempt to assess relevance of positive patch tests. COADEX stands for:

- C (Current)—Current relevance: patient exposed to drug or test agent prior to current episode of dermatitis and improves when exposure ceases.
- O (Old)—Past or old relevance of dermatitis to the test agent.
- A (Active)—Patient actively sensitized. Presents with a late reaction.
- D (Doubtful)—Relevance difficult to assess. Not known if exposure is current or not.
- E (Exposed)—History of exposure but no dermatitis; no history of exposure but a positive patch test.
- X (Cross-reaction)—Positive test due to cross-reaction with another agent.

4.2.4.5 Photopatch Testing

A photopatch test is used to investigate a drug reaction when a phototoxic or photoallergic reaction is suspected. A drug patch is applied, removed after 1 day (or on day 2 if necessary), and the skin is irradiated with 5 J/cm^2 UVA. The test is read after 2, 3, or 4 days. In performing the test, the test agent is applied to two sites, with only one being irradiated with UV light. A positive

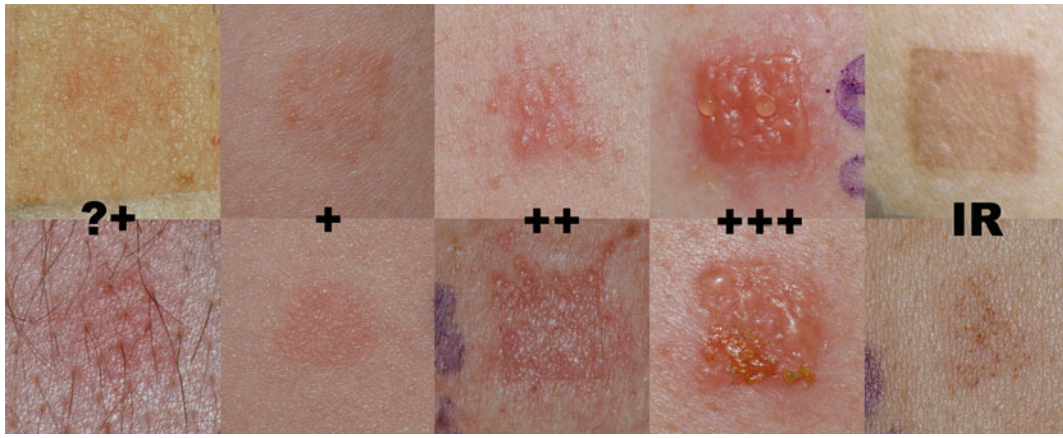

Fig. 4.5 Examples of patch test reactions showing (*left* to *right*) an equivocal positive reaction (?+); weak positive reaction (+); strong positive reaction (++); extreme reaction (+++); irritant reaction (IR). This figure should be viewed with reference to Table 4.1. From Spiewak R. Patch testing for contact allergy and allergic contact dermatitis. The Open Allergy Journal 2008;1:42. Reproduced with permission of the author

result at the irradiated site with a negative result at the nonirradiated site suggests photoallergy whereas equal positive responses at both sites suggest contact allergy.

4.2.4.6 Control Subjects

For control subjects, healthy volunteers with or without exposure to the drug can be recruited as negative controls although some investigators prefer to include dermatitis patients who proved positive to a drug but negative to the drug being investigated. Such control subjects should only be included 6 weeks to 6 months after taking the drugs, and they should enter the study with ethics approval and signed informed consent.

4.3 Serum Immunoglobulin E Antibody Tests

While the patient history and skin tests form the core of the investigative procedures for accurately diagnosing hypersensitivities to drugs, tests for the detection of IgE antibodies specific for individual drugs are a valuable diagnostic aid to supplement and confirm skin test findings, and in some cases they offer some advantages. Serum IgE antibody determinations are useful in cases of skin test-negative or equivocal reactors or when skin tests are unreliable or unavailable. A good example of their value is seen in cases where patients have a convincing history of immediate allergy to a β-lactam but a negative skin test, a discrepancy that is occasionally seen. They are also valuable for patients on certain medications that must be curtailed for skin testing, in patients with widespread skin afflictions such as eczema or psoriasis and when applied to sera taken at the time of a reaction (e.g., an anaphylactic reaction during anesthesia), before surgery, and serum taken before or after death. Until the introduction of the radioallergosorbent test (RAST) for the detection of allergen-reactive IgE antibodies in the early 1970s, skin testing and occasionally the Prausnitz-Kűstner test were the only ways of confirming a diagnosis of type I IgE-antibody-mediated disease. In its earliest form, the test was a solid phase radioimmunoassay utilizing allergen preparations attached to paper discs and a labeled second antibody to detect IgE antibodies bound to the immobilized allergens. Despite the introduction of some variations such as liquid phase systems, the solid phase technology has persisted due to improvements in the types of solid phases with enhanced allergen binding capacity, the use of monoclonal antibodies, enhanced sensitivity and accuracy, and the introduction of calibration methods for

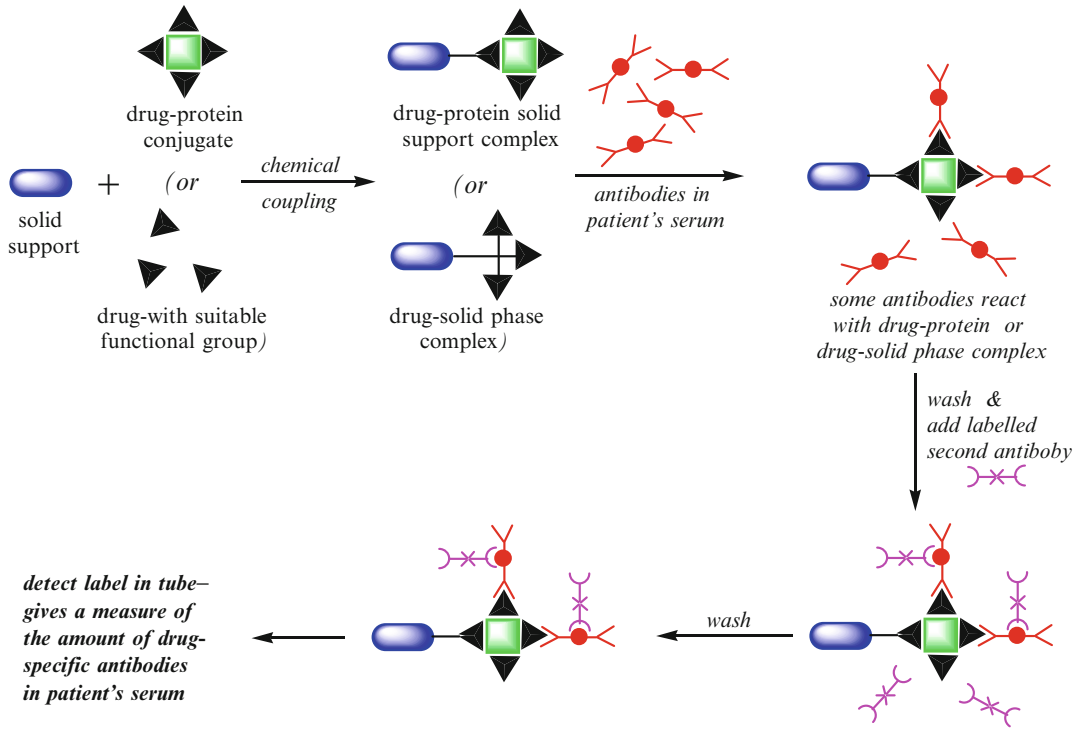

Fig. 4.6 Diagrammatic representation of the solid phase immunoassay procedure for the detection of drug-reactive IgE antibodies in serum. From Baldo BA and Pham NH. Structure–activity studies on drug-induced anaphylactic reactions. Chem Res Toxicol 1994;7:703. Reproduced with permission from American Chemical Society

the quantitative expression of results. At the same time, automation has led to greater precision and reduced turnaround times, and the addition of carefully selected nonisotopic labels and substrates has improved sensitivity and accuracy and reduced nonspecific binding. Automation and widely adopted calibration methods have also made interlaboratory standardization possible.

4.3.1 In Vitro Detection of Drug-Reactive IgE Antibodies

Specific immunoassays to detect IgE antibodies in the sera of drug-allergic patients are being increasingly used to supplement patient's histories and skin tests and increasingly valued as the range and sensitivities of tests increase. In its simplest form, protein-coupled or free drug is covalently linked to a solid phase (sometimes via a spacer arm); the complex is incubated with patient's serum, washed, and specifically bound IgE antibodies are detected with an enzyme-, fluorescent-, fluoroenzyme-, chemiluminescent-, or radiolabeled second antibody (Fig. 4.6). The biotin-avidin (or streptavidin) reaction utilizing labeled biotin can also be employed as a highly sensitive detection procedure. In preparing the drug solid phase, the drug must be immobilized but unaltered antigenically for recognition by its complementary antibodies. The chemical procedure selected to couple a drug either directly to a solid support or first to a carrier macromolecule (usually protein) and then to the support depends on the functional groups that are available on the drug or can be added to the drug. Nucleophilic addition reactions employing, for example, bis-oxirane (1,4-butanediol diglycidyl) or divinyl sulfone have proved to be widely applicable, generally not chemically destructive for the drug to be coupled and easy to carry out. Coupling employing carbodiimides is

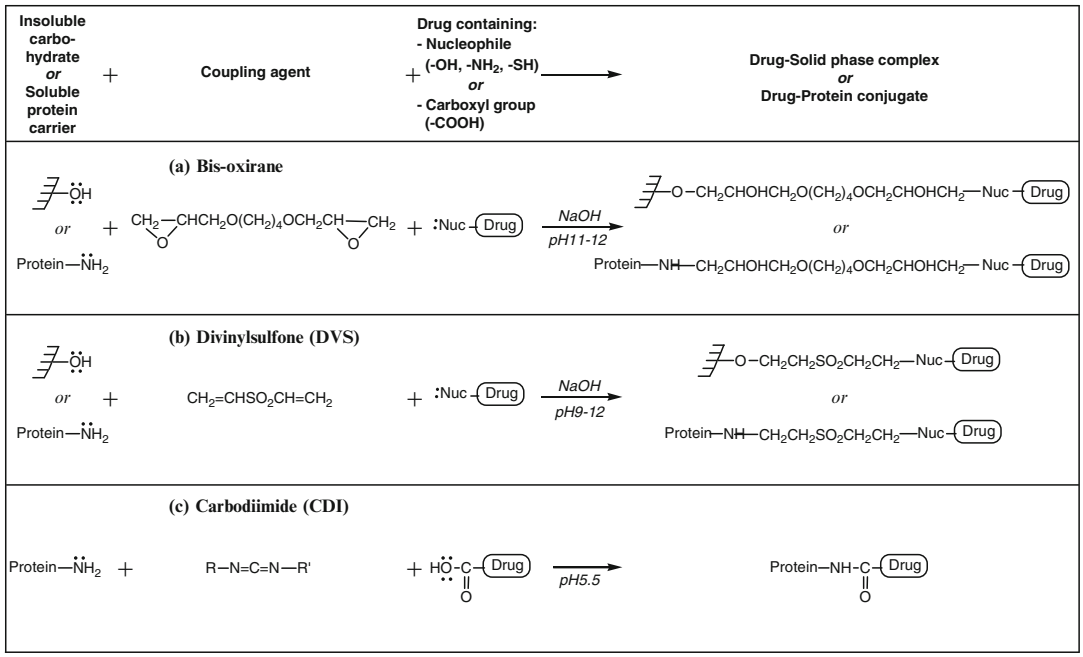

Fig. 4.7 Some widely applicable chemical strategies for the preparation of drug-carrier complexes. From Baldo BA and Pham NH. Structure–activity studies on drug-induced anaphylactic reactions. Chem Res Toxicol 1994;7:703. Reproduced with permission from American Chemical Society

also a relatively mild procedure applicable to a variety of different drugs. Carbodiimides can be used to form peptide bonds at room temperature by linking free carboxyl groups on drugs to protein amino groups (Fig. 4.7). Chemical methods can usually be applied to conjugate most drugs but sometimes it is chemically difficult and time consuming and may even involve the employment of complex synthetic steps. Sometimes a simple and convenient alternative to a complex synthesis is the substitution of suitable structural analogs that contain the identical or closely related antigenic determinant structures. This was the strategy used to prepare specific, IgE-reactive solid phases antigenically similar if not identical to the muscle relaxants succinylcholine and gallamine both of which lack suitable functional groups for easy coupling to protein or insoluble carbohydrate supports (see Sect. 7.4.3.4).

By using the binding assay in an inhibition format, the investigator can confirm specificity of the antibody-drug reaction, compare quantitatively the recognition of similar drugs, and identify the precise structures features that constitute the drug allergenic determinants (Fig. 4.8). Inhibition assays are indispensable in the study of immediate allergic reactions to drugs not only to check specificity but also for cross-reactivity and allergen structure investigations and to help bridge the clinic-laboratory divide by relating and correlating, on a quantitative basis, allergic recognition in the test tube and in the patient. Although this correlation has not always been as good and as informative as one would hope, improvements in assay specificities and sensitivities and insights gained from increased knowledge of precise allergenic structures and their recognition promise to significantly increase the value and utility of drug-IgE antibody measurements. So far in clinical allergy practice and research, quantitative immunochemical approaches have been largely ignored and interpretations of allergic recognition and sensitivity have been based on undoubtedly clinically relevant skin tests and other tests that are not strictly quantitative.

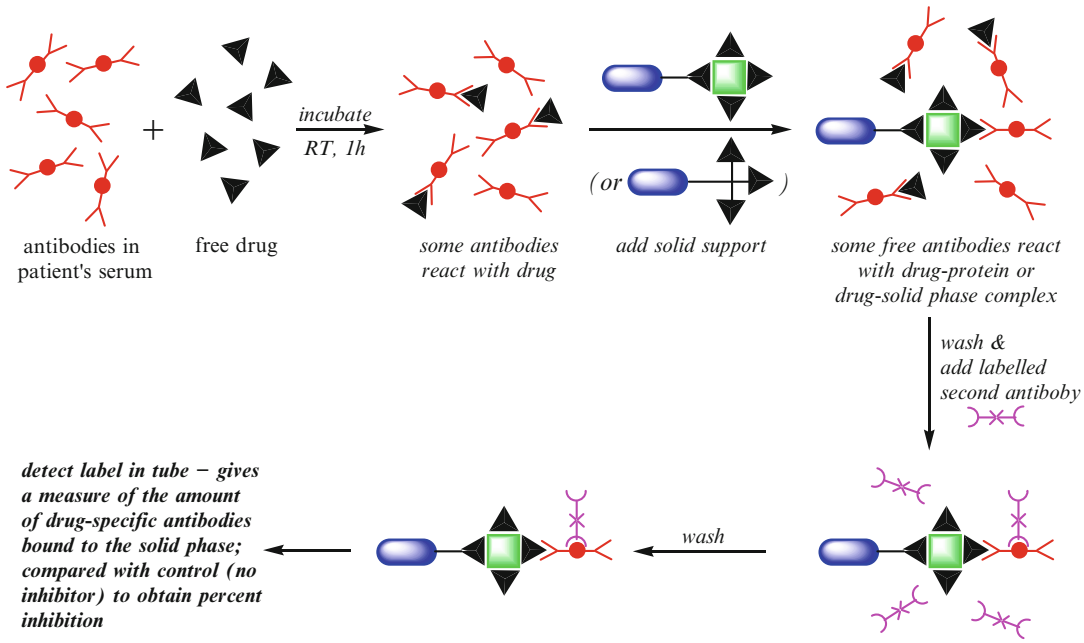

Fig. 4.8 Diagrammatic representation of the solid phase immunoassay inhibition procedure used in quantitative hapten inhibition studies for the establishment of specificity of antibody binding and for the identification of drug allergenic determinants. From Baldo BA and Pham NH. Structure–activity studies on drug-induced anaphylactic reactions. Chem Res Toxicol 1994;7:703. Reproduced with permission from American Chemical Society

4.3.2 Tests for the Clinic and Research

For routine application of immunoassays to detect drug-reactive IgE antibodies, the current situation is characterized by the restricted availability of specific drug tests. Perhaps the only suitable standardized testing agents that can be accessed widely are the Phadia, now Thermo Scientific, ImmunoCAP® small range of drug solid phases comprising penicilloyl G, penicilloyl V, amoxicilloyl and ampicilloyl determinants, cefaclor, chlorhexidine, chymopapain, gelatin (bovine), insulin (human, bovine and porcine), pholcodine, morphine, and succinylcholine. For research purposes only, ACTH, protamine, and tetanus toxoid are offered. Each ImmunoCAP® is described as a capsule enclosing a cellulose derivative with a high binding allergen capacity per mg of cellulose. The ImmunoCAP® testing agents and format are now backed by a large number of publications focusing on performance and clinical utility making this allergy test system the best described and most studied one available but, even so, the range of available tests for individual drugs remains inadequate. In fact, at the research level, a relatively wide range of drug solid phases have been prepared and successfully used to identify drug-reactive IgE antibodies in allergic patient's sera and, in some cases, to identify allergenic structural determinants. Results with some of these "in-house" tests have been at least as good and sometimes superior to the corresponding commercial assays. Details for the preparation of drug solid phases and procedures for the assays utilizing them have been published for the following drugs:

Penicillins (penicilloyl and penicillanyl determinants of benzylpenicillin, phenoxymethylpenicillin, ampicillin, amoxicillin, ticarcillin, flucloxacillin, and cloxacillin) (Chap. 5); cephalosporins (cefaclor, cephalothin, cefalexin, ceftazidime, ceftriaxone, cefuroxime, cefotaxime, cefadroxil) (Chap. 5); tetracycline and doxycycline; sulfamethoxazole;

trimethoprim; chlorhexidine; quinolones (ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, pipemidic acid, rufloxacin) (Chap. 6); neuromuscular blocking drugs (morphine for the detection of members of the group, alcuronium, gallamine, rocuronium, succinylcholine, d-tubocurarine, vecuronium); thiopentone (Chap. 7); local anesthetics (mepivacaine, procaine) (Chap. 7); opioids (morphine, codeine) (Chap. 8); aspirin, propyphenazone, and other pyrazolones (Chap. 9); ioxaglic acid (Chap. 10); a number of monoclonal antibodies (Chap. 11); methylprednisolone succinate ester (Chap. 12); *L*-asparaginase (Chap. 13).

Many of these assays essentially remain as unstandardized research tools but each one has produced results that suggest further investigation, refinement and steps to achieve validation are worth considering. A major obstacle in the introduction and development of any new test for the detection of drug-reactive IgE antibodies is the availability of a sufficient supply of reactive sera from allergic patients. This problem is particularly acute when the drug is an infrequent cause of type I allergy. In cases where the development of a new test is considered desirable, when sufficient sera from allergic patients is available and the necessary chemical procedures have been devised, a series of investigative steps to determine specificity of the assay are obligatory. These essential controls and procedures are set out in Table 4.2.

Table 4.2 Necessary procedures and criteria to be satisfied for the detection of drug-specific IgE antibodies

A. Requirements for assay
1. Serum from allergic subjects
2. Drug (or close structural analog) covalently linked to suitable solid phase
3. Monospecific, affinity-purified anti-human IgE antibodies tagged with suitable reporter group (e.g., radioisotope, enzyme, fluorescent label, colloidal metal particles, etc.)
4. Detector—spectrometers, spectrophotometer, etc.
B. Controls
1. Free solid phase
2. Solid phase covalently linked to
(a) Structurally related compounds
(b) Structurally unrelated compounds
3. Sera from nonallergic ("normal") subjects
4. Serum from cord blood (IgE-"free" control)
5. Sera from subjects allergic to common allergens; include sera with high total IgE levels
C. Demonstration of specificity
1. Inhibition of binding of IgE antibodies in subject's serum to drug solid phase by preincubation with free drug
2. Inhibition with structurally related compounds
3. No inhibition with structurally unrelated compounds
4. Binding of IgE antibodies to solid phase covalently linked to structurally related compounds

4.3.3 Quantitation, Interpretation and Reporting of Results

It is obviously desirable to be able to detect IgE antibodies in very sensitive, specific, and accurate assays and to report the results in quantitative terms. Over the last two or three decades, a variety of methods for quantification have been developed. Many laboratories have reported results using a class or scale system ranging from class 0, no reaction to class one, a low level of specific IgE and so on to class six, an extremely high level. Although such a semiquantitative scale helps to interpret, more finely discriminate, and sort the relative strengths of the different reactions, the classes are often wide, use different calibrations, and differ between different test systems. The traditional performance characteristics of sensitivity and specificity for tests measuring specific IgE antibodies simply divide the results into positive and negative with a cutoff that is often difficult to interpret clinically. This is most easily seen when low levels of IgE occur with unclear or vague clinical symptoms. Receiver-operating characteristic (ROC) curves provide more information to aid discrimination between positive and negative results but decision thresholds are not displayed and they are difficult to apply to small samples. The concentration of IgE antibodies assessed by immunoassay is related to the presence of allergic symptoms indicating that a quantitative measurement of antibodies will yield more informative results than a simple positive or negative answer. This relationship between clinician-diagnosed positive and negative findings

and the quantitative levels of specific IgE was analyzed using a logistic regression model. Without a fixed cutoff, the logistic model showed better agreement between IgE antibody levels and clinical disease than could be obtained with the conventional sensitivity and specificity approach. This quantification demonstrating a link between specific IgE antibodies and allergic reactions helps to achieve greater diagnostic accuracy.

The best calibration system for specific IgE antibodies currently in use is based on the World Health Organization 75/502 IgE standard using a multipoint calibration curve. A number of clinical IgE assays, including the ImmunoCAP® (Thermo Scientific) assays, have a working range from 0.1 to 100 kU_A/l, where A represents the amount of allergen-specific IgE antibody in the sample and 1 $kU_A/l = 1$ KU/l = 2.42 μg/l. Note that although different assay systems may present their results in the same units, viz. kU_A/l, this does not guarantee that the results are correct and interchangeable. This is probably because most allergen preparations contain a mixture of different individual allergens with different allergenic potencies and the compositions of the allergen preparations used may vary between manufacturers. One might predict that the situation with drugs should be simpler and more predictable since the allergenic material employed in the assay is a pure, standardized drug that can be coupled to the solid support using well-defined chemical procedures that can be followed by all manufacturers to produce a standardized test solid phase.

Special care should be exercised when results for drug-specific IgE antibodies fall below the limit of quantitation and are undetectable. Although this may indicate a nonsensitized individual, the finding might be due to the presence of a hypersensitivity state without IgE involvement or the blood sample might have been taken too long or too soon after the adverse reaction. In the former case, antibody levels may have decreased over time while in the latter case, drug-reactive antibodies may have been largely consumed with no time for their replacement.

4.4 Drug Challenge (Provocation) Testing

In the diagnosis of drug hypersensitivity reactions, a drug challenge, or provocation test, is the controlled step-wise administration of a drug in a supervised hospital environment in order to determine if the drug was the causative agent in a patient's allergic reaction. The challenge test is also used to determine if a particular drug can be safely administered to a patient. In the ideal diagnostic test for a drug-induced hypersensitivity reaction, challenge with the suspected drug reproduces the identical clinical symptoms and signs of the original or so-called index reaction. For this reason, the test is the best way to confirm an allergic reaction, and it is considered to be the "gold standard" in the diagnosis of drug hypersensitivity reactions. In fact, a positive challenge test will not only produce the symptoms of hypersensitivity but also those of other adverse responses regardless of the mechanism. The benefit of the challenge test is obvious when one considers that a positive result makes the need for allergen avoidance clear and unequivocal while a negative result gets rid of the incorrect and unnecessary classification of the patient as hypersensitive to the drug. If a reaction does occur upon challenge, it is likely to be milder because of the slow and incremental dose escalations. Drug challenge tests are particularly important when other usually employed tests, particularly skin tests, are not available or possible, for example, with histamine-releasing drugs such as codeine; where sensitivity is lacking with non-β-lactam antibiotics; with glucocorticoids and heparins; and with drugs such as local anesthetics (see Chap. 7), NSAIDs in patients with the cross-reactive pattern (Chap. 9), and contrast media (Chap. 10) that may produce unreliable results. A challenge test is also a reliable way to check on a previous test result such as a skin test or serum IgE antibody finding. If these tests do not lead to a conclusive result, the challenge test may be the only way to achieve a diagnosis.

Before undergoing challenge, patients should be presented with a consent form to read and sign

if they agree to go ahead with the procedure. The form should state the purpose of the test, set out the procedures involved, and summarize the benefits and risks. Some practitioners believe that drug challenge tests should be performed at least 4 weeks after the drug reaction, but there is no general agreement on this or on any upper time limit. Antihistamines should be discontinued at least 5 days before the scheduled appointment and the administering clinician should be advised if beta-blockers or ACE-inhibitors are being taken or if the patient is pregnant. The patient's health should be good on the day of testing with no sign of allergy or virus infection. Checks on blood pressure, pulse, peak expiratory flow, and oxygen saturation levels may be done before, during, and at the conclusion of the test. Challenge can be oral, parenteral (subcutaneous, intramuscular, or intravenous), bronchial, nasal, cutaneous, or conjunctival depending on the specific reaction and drug. For drugs that are ingested or injected, the oral route is preferred. Provocation is commenced with a small dose of the drug, and this is gradually increased at 30-min intervals provided no adverse reaction occurs after the previous dose. This procedure is continued until the desired dose (usually the dose that would be prescribed or the total daily dose) is reached. Table 4.3 lists a range of commonly used drugs, their usual daily dosages, and increasing challenge doses administered during provocation testing. For details of oral and parenteral provocation testing for penicillins, see Chap. 5, Table 5.3. If any adverse reaction that looks like an allergic response occurs, for example, wheezing, swelling of the throat, a drop in blood pressure, or rash, the challenges are discontinued and the test interpreted as positive, in other words, the patient is judged allergic to the drug. Minor symptoms like itching and some redness might not be considered a sufficient reason to curtail the test but, if this is done, oral antihistamines are usually enough to control the reactions. Severe reactions are treated promptly with epinephrine and/or other medications such as steroids, bronchodilators, antihistamines, and, if necessary, intravenous fluids. Placebo-controlled drug challenges may be either single blind, when the patient is given the drug and a placebo without knowing which is which for each dose or double blind when both the patient and the clinician are unaware whether and when the drug or placebo is given. The time taken for drug challenge tests can vary widely depending on the drug, the possible severity of any allergic reaction it might elicit, and the slow or rapid graded nature of dosage. Challenges may be as short as 2 or 3 h or extended over many hours or even days. After completion

Table 4.3 Doses of some commonly used drugs employed in provocation testing

Drug	Dosage amounts (mg) and sequence[a,b]	Usual daily adult dose[c] (mg)
β-Lactams[d]		
Cefaclor	1, 5, 25, 125, 500	750
Cefazolin[e]	1, 5, 25, 100, 500, 2,000	1, 500–3,000
Cefuroxime	1, 5, 20, 80, 400	500
Macrolides		
Azithromycin	1, 5, 25, 57, 125, 250	500
Erythromycin	1, 5, 25, 100, 500, 1,500	2,000–3,000
Quinolones		
Ciprofloxacin	1, 5, 25, 100, 500	500–1,500
NSAIDs[f,g]		
Ibuprofen	1, 5, 20, 80, 150, 300	200–1,200
Diclofenac	1, 5, 20, 80	100–150
Piroxicam	1, 3, 6, 10	20
Acetaminophen	1, 10, 50, 250, 500, 1,000	500–4,000
Steroids		
Prednisolone	2, 10, 20, 40	20–80
Betamethasone	0.2, 1, 2, 4	3–12
Proton pump inhibitors		
Omeprazole	1, 5, 10, 20	20–40
Local anesthetics		
Lidocaine[h]	2, 20, 40	20–60

Doses administered orally unless indicated
Doses taken from Messaad D et al. Ann Intern Med 2004;140:1,001
[a] For anaphylactic shock patients start with 1/10th of the dose shown here
[b] Doses administered at 30 min intervals
[c] Recommendations of the French Agency on Drug Safety (http://www.AFSSAPS.sante.fr)
[d] For penicillin provocation testing see Table 5.3
[e] Doses administered IV
[f] NSAIDs—nonsteroidal anti-inflammatory drugs
[g] For aspirin provocation testing see Sect. 9.5.2.1.3.1
[h] Administered subcutaneously using solution 20 mg/ml

of the test, patients who did not develop a reaction are kept under observation for 1 h and patients with minor reaction are observed for 2 h. Overnight stay in hospital should follow a serious allergic reaction.

Some variations in procedures are needed for some drugs. With aspirin, (acetylsalicylic acid) for example, the interval between doses is usually longer and of the order of 60–120 min rather than 30 min (see Sect. 9.5.2.1.3) and a starting dose of 40–60 mg is proposed for patients with aspirin-exacerbated respiratory disease if they are given a leukotriene-modifying drug. In order to confirm the absence of allergy (particularly of the delayed type) to some drugs, for example, antibiotics, patients who tolerated the test are sometimes sent home with a 5–7 day course of the drug. With the β-lactams, 4–9 % of cases confirmed by challenge testing have a delayed reaction demonstrated by a positive intradermal or patch test. With noncooperative young children, drug challenge tests with β-lactams have proved to be well tolerated.

In general, drug challenge tests should not be performed on pregnant women, on patients with uncontrolled asthma, acute infections, and some diseases involving the major organs like the heart, lung, liver, and kidneys. It should never be performed on patients who have experienced SJS, TEN, DRESS, exfoliative dermatitis, vasculitic syndromes, and life-threatening immunocytotoxic reactions.

Apart from the obvious diagnostic value and benefits for the patient that a drug challenge can bring, it remains a serious and potentially dangerous procedure to be used with great caution. It should not be used without careful analysis of the possible benefits for the patient, the particular drug hypersensitivity situation, and the patient's state of health. Essentially, provocation with a suspected drug can be undertaken when it is judged that the risk of a serious allergic reaction is low while the value and quality of information gained to aid the patient is high. In other words, performance of this test, more than the other tests discussed here, comes down to a decision after weighing up the risk-to-benefit ratio. If for some reason a high risk remains but it is judged that the benefits warrant proceeding, challenge can be undertaken in an intensive care unit for safety reasons. For patients with a history of anaphylactic shock, intravenous catheters should be in place for the duration of the test. After discharge, there is the possibility of a delayed allergic reaction as part of a biphasic response. This can occasionally be lethal so at the time of discharge the patient should be provided with adequate emergency treatment of antihistamines, steroids, or inhalers.

4.5 Detection and Measurement of Released Mediators/ Markers of Hypersensitivity

A wide and chemically varied range of biologically active agents may be released during drug-induced adverse reactions in humans. Mast cells and basophils are the main cells activated during a type I IgE antibody-mediated reaction. Preformed mediators of allergy including histamine, the enzymes tryptase, carboxypeptidase, chymase and cathepsin G, serotonin, platelet activating factor, and eosinophil and neutrophil chemotactic factors are released from the granules of mast cells while potent lipid mediators such as leukotrienes LTB_4, LTC_4, and LTD_4; prostaglandin PGD_2; and thromboxanes are newly synthesized. Cytokines, including IL-4 and IL-13 that stimulate Th2-cell responses, IL-3, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF), and chemokines such as CCL3 and CCL5 (RANTES) are also released. Of this large, assorted group of liberated mediators promoting such a diverse range of physiological and pharmacological effects, only histamine, the enzyme tryptase, and to a lesser extent the leukotrienes have so far found any diagnostic application to drug allergies.

4.5.1 Tryptase

4.5.1.1 Tryptase Genes

Although many, if not most, of the autacoids, enzymes, biologically active lipids, and various other factors involved in the inflammatory cascade

following mast cell activation contribute to the clinical manifestations of an allergic reaction, there is no evidence that tryptase has such a role. Tryptases are neutral proteases belonging to a subgroup of the trypsin family serine peptidases. Almost all human mast cells make and store tryptases which constitute the major proteins in the secretory granules. Basophils, which seem to have a lesser role in anaphylaxis than mast cells, make far smaller amounts of tryptase. Four gene loci on chromosome 16 encode the human mast cell tryptases, and additional diversity is added by α, β, γ, and δ allelic variations. The gene sites and alleles they harbor are termed TPSAB1 (α and β1 alleles), TPSB2 (β2 and β3 alleles), TPSD1 (δ alleles), and TPSG1 (γ alleles). δ-Tryptase and γ-tryptase are not found in serum; the biological role of δ-tryptase is not known but γ-tryptase is an active peptidase that stimulates IL-13 production and induces bronchial hyperresponsiveness in mouse airways. Only α and β tryptases make up the circulating tryptase and only these two forms show a relationship to anaphylaxis. Considering only the α and β alleles, three genotypes are possible αα:ββ, αβ:ββ, and ββ:ββ.

4.5.1.2 α and β Tryptases

Circulating tryptase levels are now known to consist mainly of inactive β-protryptase with perhaps a small amount of α-protryptase. In fact, total tryptase levels in serum are not significantly different in subjects with and without the gene for α-tryptase. α-Tryptase is not stored in granules but is instead secreted as an inactive proenzyme. This means that upon degranulation, α-tryptase is not part of any increase in serum tryptase levels and is therefore not a useful marker of mast cell activation and anaphylaxis. Tryptase, stored in secretory granules and released during activation of mast cells, unlike protryptases, is not spontaneously secreted by resting mast cells and is referred to as mature tryptase. In skin and lung mast cells most, if not all, of the mature tryptase is β-tryptase. Thus, α and β protryptases are spontaneously secreted by resting mast cells while mature β-tryptase is stored and released upon degranulation of the mast cell. Mature tryptase levels in blood therefore reflect, and are a measure of, mast cell activation.

4.5.1.3 Tests for Mature and Total Tryptase

The immunoassay result for *total* tryptase reflects the mast cell number and measures pro and mature forms of α and β tryptases. Protryptases account for nearly all of the tryptase in normal sera which show a normal level of approximately 5 ng/ml and a range of 1–15 ng/ml. Results of a study of 126 apparently healthy individuals 12–61 years old with the Phadia ImmunoCAP Tryptase (Thermo Scientific) commercially available fluoroimmunoenzymatic assay showed a geometric mean of 3.8 ng/ml and a 95th percentile of 11.4 ng/ml. From the pioneering studies of L. B. Schwartz and coworkers, blood samples (to obtain serum after clotting) should be taken 15 min to 3 h after the suspected reaction, although significantly raised tryptase levels can usually be detected up to 6 h (and sometimes more) after the reaction. Normal levels return 12–14 h after the initial release. For shipping, serum samples can be kept at room temperature for 2 days otherwise samples are stored at 4 °C for 5 days or −70 °C for longer periods. Total tryptase levels greater than 20 ng/ml are generally found in patients with systemic mastocytosis. For postmortem examination of tryptase levels, blood can be taken up to 24 h after death although results from at least one investigation suggest that blood samples taken after a significantly longer time interval can still be successfully examined to yield important diagnostic information. In a study of two fatal cases of perioperative anaphylaxis employing immunoassays for neuromuscular blocking drugs together with the tryptase assay, highly elevated tryptase levels (compared to levels in preoperative blood samples) found in blood taken 48 and 72 h after death were matched by the clear-cut detection of IgE antibodies to thiopentone and succinylcholine.

The assay for *mature* tryptase is a capture assay using a monoclonal antibody that recognizes mature α and β tryptases although β-tryptase constitutes the major, if not exclusive, form of mature tryptase in blood. Normal levels of mature tryptase in serum are less than 1 ng/ml whereas

Table 4.4 Total and mature tryptase levels in serum of normal subjects and patients with anaphylaxis or systemic mastocytosis

Subject	Total tryptase level (ng/ml)	Mature tryptase level (ng/ml)	Ratio of total to mature levels
Normal	1–15	<1	–
Acute systemic anaphylaxis	>Baseline	>1	<10
Nonacute systemic mastocytosis	>20	<1[a]	>20

Data from Schwartz LB. Immunol Allergy Clin N Am 2006;26:451

[a]Sometimes small elevations

levels equal to or above 1 ng/ml indicate mast cell activation. Three or four hours after the onset of anaphylaxis, levels generally revert to less than 1 ng/ml. In anaphylaxis, the ratio of total to mature tryptase is typically less than 10 while in systemic mastocytosis the ratio is generally more than 20. Mature tryptase levels greater than 10 ng/ml in postmortem samples suggest that systemic anaphylaxis might have occurred (Table 4.4). The mature tryptase assay is only available in the laboratory of Dr Lawrence Schwartz, Medical College of Virginia, Richmond, VA, USA.

4.5.1.4 Tryptase and Drug Allergy

From the time of its general introduction in the early 1990s, the tryptase assay has gone a long way in helping to answer the question "is this drug reaction anaphylaxis?" From the beginning, the test proved valuable for selecting an homogenous population to evaluate tests for dug allergy; initial screening with the tryptase test sometimes eliminating a significant number of patients from subsequent skin and IgE antibody testing. Drug-reactive IgE antibody tests on sera taken at the time of the reaction may identify an immune basis for the reaction but such tests are not always available and some assays in their current form may give false negatives to some drugs. At first site, the determination of plasma histamine concentrations can be valuable in demonstrating an anaphylactoid reaction, but these tests can be technically and logistically difficult with samples needing to be obtained preferably within 10 min of the reaction, a time when resuscitation is a priority. Early studies with the assay showed that increases in mast cell tryptase concentrations in serum seemed to occur only in immunological reactions but this proved to be not invariable—increased tryptase concentrations may occur with direct histamine release. A good reported example of this is an extremely high serum tryptase level in a patient who died after receiving a bolus of vancomycin. In cases of life-threatening drug reactions during anesthesia where neuromuscular blocking drugs are known to be the biggest cause of anaphylaxis and where research is more advanced than in many other areas of drug allergies, the tryptase test has become part of the established and standard protocol along with skin tests and tests for serum IgE antibodies for diagnosing and establishing the mechanism of drug-induced reactions. It is concluded that increased mast cell tryptase concentrations are a valuable indicator of an anaphylactic reaction during anesthesia and although elevated levels favor an IgE-antibody-mediated cause, they do not always distinguish between an anaphylactic and an anaphylactoid reaction.

4.5.2 Histamine

The presence and biological role of histamine in immediate and some other hypersensitivity reactions is well established. Histamine release from human blood leukocytes after challenge with drug in vitro is occasionally employed but in general, for diagnostic purposes, histamine concentrations in biological fluids have rarely been routinely measured. The reasons for this in the diagnosis of drug allergy are not hard to find: the half-life of histamine in plasma is short (approximately 1–2 min) due to its rapid methylation by histamine methyltransferase and oxidation by diamine oxidase; blood sampling after a reaction, especially in an emergency situation like anaphylaxis, is difficult and has severe time constraints; false positive results are often assumed to be likely following disruption of cells, in particular basophils, during blood sampling and handling; and assays have commonly had technical and practical shortcomings.

4.5.2.1 Histamine Concentrations in Blood

The threshold for pathological levels of histamine in plasma is said to be 1 ng/ml or 9 nmol/l while levels greater than 10 ng/ml (90 nmol/l) cause serious cardiovascular sequelae. Plasma histamine levels in normal subjects are generally less than 9 nmol/l when measured by the fluorimetric method. Isotope dilution mass fragmentography gave a range of 0.8–3.6 nmol/l, and the monoclonal anti-acylated histamine-based radioimmunoassay employed in a number of allergy-related studies has shown concentrations of 3.33 nmol/l ($n=14$), 0.193 (1.7 nmol/l) ± 0.08 ng/ml ($n=40$) and 0.8 ± 0.4 nmol/l ($n=13$) in normal subjects and 1.63 ± 0.61 nmol/l ($n=35$) in anesthetized patients. Histamine was measured 15 min after induction of the latter patients none of whom had any adverse reactions. The normal histamine concentration represents less than 0.5 % of the histamine concentration in blood. Histamine in blood and in plasma is generally said to be unstable but precise figures on its half-life under normal and varied conditions are hard to find. Studies on six normal volunteers showed the half-life of infused histamine to be 102 s. A comparison of the metabolism of infused histamine in urticarial patients, normal subjects and atopics revealed half-lives of 6.2 ± 1.3 min, 4 ± 0.7 min, and 3 ± 1.2 min, respectively.

4.5.2.2 Measurement of Histamine

At least until the late 1980s, assays for histamine were often difficult to undertake and lacking sensitivity and specificity. Some methods for measuring histamine, for example, the automated fluorimetric assay developed by Siraganian, show good sensitivity and specificity, but the method is somewhat complicated and technically demanding and has therefore not been employed in a large number of laboratories worldwide. Immunoassays for histamine in RIA or ELISA format are available commercially from a number of different companies. They are more accessible and easy to use than many of the older assays and some with good performance characteristics have been adopted as a diagnostic tool. For drug allergy studies, aliquots of diluted whole blood are challenged with drug in vitro at 37 °C, supernatants are frozen, and the histamine content is measured after cell lysis. Anti-IgE can be added to assess releasibility of histamine from basophils, and the total histamine concentration of the cells is measured after cell lysis. The figure for spontaneous histamine release is subtracted from the drug-induced release and total histamine results. Spontaneous release should normally be less than 5 % of the total histamine content of blood (20–200 ng/ml). Histamine release greater than 5 % of the total histamine content (after subtracting the spontaneous release figure) is regarded as a positive drug-specific result. In an effort to investigate the potential of measuring released plasma histamine for the diagnosis of drug allergies and the possible problems associated with the necessary measurement procedures, a radioimmunoassay utilizing a monoclonal antibody specific for succinyl glycinamide derivatized histamine was utilized. This work is mentioned since it covers most of the important technical points relevant to measuring histamine in plasma and highlights the all-important question of the mediator's stability. The assay has cross-reactivity ratios for *N*-methylhistamine and histidine of 1/14,500 and 1/250,000, respectively, and a claimed sensitivity of 0.2 nmol/l, although it proved to be 0.5 nmol/l with a variation coefficient of 10 % in this study. A range of seemingly small but essential investigations of aspects of the methodology of sample collection, handling, and storage as well as stability studies were undertaken, sometimes with surprising results. Many of the precautions thought to be necessary, and taken, when using the fluorimetric assay were found to be unnecessary for the radioimmunoassay. This was demonstrated in experiments on the sampling procedure, leakage of histamine from basophils at room temperature and 4 °C, and freeze-thawing of samples. Of most interest, were the findings on the stability of histamine in blood and serum samples. The in vivo half-life of infused histamine in plasma has been estimated to be between 1 and 2 min but in 12 patients with increased plasma histamine concentrations during anaphylaxis, all had increased plasma histamine concentrations in the first sample taken

5–60 min after the onset of the reaction. The mean histamine concentration in the second sample taken between 30 and 200 min after the first sample was significantly less but also still elevated. All but one of the 12 patients still had increased plasma histamine concentrations at pathological levels 60 min after the onset of the reaction. In the anaphylactic patients, these results point to a half-life of histamine significantly higher than 1–2 min—in fact, a figure closer to 20 min is likely. This prolonged half-life may be a reflection of the extended release of histamine or saturation of the enzymes involved in its metabolism. In vitro, histamine concentrations did not change significantly in whole blood left at room temperature for 2 h or overnight at 4 °C or in separated plasma after 48 h at room temperature or 72 h at 4 °C. In previous in vitro studies, histamine has been reported to be stable for 30 min at room temperature and at 4 °C when added to normal plasma. The stability of histamine in patients with normal metabolism was in contrast to results obtained with plasma from heparinized patients and from pregnant women, the latter apparently due to increased diamine oxidase activity during pregnancy. Of interest was the finding that histamine disappeared from plasma at a slower than expected rate during anaphylaxis indicating that there is still likely to be time for blood sampling after emergency treatment. This has led some investigators to conclude that histamine levels in plasma should be determined in cases of drug-induced anaphylaxis and to go so far as to state that there is no justification, apart from cost, for not doing so.

4.5.2.3 Histamine in Drug Allergy Diagnosis

It is safe to say that the above belief is not widely held and the conclusion not widely supported. An investigation of the predictive capacity of histamine release for the diagnosis of drug allergy using the sensitive anti-acylated histamine immunoassay found that net histamine release was positive in only 18 of 35 drug-allergic patients (median total histamine 61 ng/ml), 12 of 33 allergic patients with no drug allergies (50 ng/ml), and 15 of 40 controls with no history of drug allergy (55 ng/ml). Overall, the sensitivity (51.4 %), the specificity (63 %), and the positive predictive value (29.3 %) were poor, but the negative predictive value (81.1 %) was judged to be of value for ruling out some reactions that appeared to be allergic. On the basis of these results, one could not advocate histamine release as a routine test for the diagnosis of drug allergies but it seems premature to accept this conclusion as final and generally applicable to drug allergy for a number of reasons. More extensive studies are needed with homogenous populations of patients particularly with regard to the type of hypersensitivity, the drugs involved, and the time interval between the reaction and testing. Patients with any one of the four commonly recognized types of hypersensitivity may be classified as drug allergic and testing may compare time intervals of as little as a month to a number of years. There are also indications that histamine release tests might be of more value for some classes of drugs. With neuromuscular blocking drugs, diagnostic applications of histamine release tests have yielded encouraging results. One study, for example, involving 40 patients allergic to muscle relaxants and 44 controls, showed a sensitivity of 65 % and specificity of 100 %. Some encouraging results have also been obtained with β-lactams, especially in the case of rapid onset reactions. It, therefore, seems too early to leave histamine release tests out of any serious consideration of drug allergy diagnosis. Too few well-designed and executed investigations have been undertaken so far and it seems true to say that some of the expected difficulties of working with histamine are more assumed than real.

4.5.3 Cysteinyl Leukotrienes

For the structures of the cysteinyl leukotrienes and a discussion of their biosynthesis and role as inflammatory mediators, see Sect. 3.2.5.2.

The cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4, sometimes called sulfidoleukotrienes, are a family of potent bioactive peptide-conjugated lipids formed by mast cells, basophils, eosinophils, neutrophils, macrophages, dendritic cells,

and T lymphocytes. Basophils produce more than 100 times the amount produced by eosinophils. Following allergen-induced cross-linking of IgE antibody receptors on mast cells, cysteinyl leukotrienes are released newly synthesized within minutes. Well recognized for their powerful bronchoconstricting effects and exacerbating asthma, these lipid mediators are found in bronchoalveolar lavage fluid and nasal secretions from atopic human subjects after allergen exposure. As an indication of potency, LTE_4, for example, is considerably more powerful than histamine in decreasing airflow.

4.5.3.1 Tests for Allergen-Induced Release of Cysteinyl Leukotrienes

The release of cysteinyl leukotrienes from isolated peripheral blood leukocytes following allergen challenge in vitro has been utilized as a test for immediate hypersensitivity in the form of the Bühlmann CAST® (Cellular Allergy Stimulation Test) assays offered commercially as CAST® ELISA, a microtiter plate ELISA immunoassay, and as a flow cytometric assay Flow CAST® (Bühlmann Laboratories AG, Schönenbuch, Switzerland). Before the CAST® ELISA test is carried out, patients should discontinue treatment with antihistamines, corticosteroids, or chromoglycic acid 3–7 days prior to sample collection. Blood is collected in the presence of EDTA and stored at 2–8 °C for up to 24 h if necessary before 200 µl is taken for leukocyte isolation by dextran sedimentation of red cells. After resuspension of leukocytes, aliquots in stimulation buffer are taken for challenge with allergen and to serve as positive control (antibody to high affinity IgE receptor FcεRIα) and background spontaneous release tubes. After incubation for 40 min at 37 °C and centrifugation, cell-free supernatants along with standards (range 50–3,200 pg/ml) and controls are added to pre-coated microtiter wells for the determination of de novo synthesized leukotrienes (LTC_4, LTD_4, and LTE_4) in a competitive immunoassay at room temperature. The ELISA utilizes alkaline phosphatase for color development. The whole procedure is said to take5½ h and an analytical sensitivity of 19 pg/ml is claimed. Addition of IL-3 is said to increase cysteinyl leukotriene release induced by allergens although some investigators believe that IL-3 may stimulate basophils causing false positives, high backgrounds, and nonspecific reactions. In testing for drug hypersensitivity, the addition of IL-3 is said by some to be essential to obtain sensitivity. $C5_a$, a nonspecific activator of basophils and thought to enhance allergen stimulation, has been added in the CAST® assay but its use seems to be not widely supported. In the absence of fresh cells from patients, successful passive sensitization with serum would be an important added feature of the CAST® assay. However, investigations so far have shown that it is less sensitive than when untreated fresh cells are used. In CAST®-ELISA assays, up to 6–8 % of cells have been reported to be nonresponders. This has been said to be due to reconstitution of the lyophilized anti-IgE reagent in solutions with a suboptimal Ca^{2+} concentration.

The determination of cysteinyl leukotrienes by ELISA in cell supernatants has been combined with flow cytometric examination (see Sect. 4.6) of CD63 expression on basophils in the Flow CAST® assay. Gating of basophils is achieved with the aid of the constitutively expressed eotaxin receptor CCR3 detected with a phycoerythrin fluorescence-labeled monoclonal antibody. CD63, detected with a fluorescein isothiocyanate (FITC) fluorescence-labeled monoclonal antibody, is utilized as the cell surface activation marker expressed on sensitized degranulating basophils. Positive controls include a monoclonal antibody to cross-link the FcεRIα receptor and the tripeptide chemoattractant *N*-formyl-methionine-leucine-phenylalanine (fMLP) that activates basophils nonimmunologically. The basophil surface expressed antigen CD203c detected with a different fluorescence-labeled antibody can also be used as an extra activation marker. Both markers are used in the Flow CAST® highsens commercially available kit. There are reports of 8–10 % of nonresponders in some Flow-CAST® studies.

When considering the cellular antigen stimulation and basophil activation tests, an interesting point to bear in mind is the sensitivity of CD63 expression to external Ca^{2+} concentrations compared to the calcium requirement for LTC_4

production. In addition, calcium sensitivity may vary from one individual to another. The consequence of this can be a positive CAST® result and a negative basophil activation test (BAT) result.

4.5.3.2 Correlations of CAST® with the Clinical Situation and Other Diagnostic Tests

These are not always clear-cut and uniformly good. Good correlation has been reported with the severity of allergic rhinitis reactions but not for asthma severity. For β-lactam drugs, CAST® was shown to be positive only in cases of anaphylaxis but another group reported positive findings in cases of β-lactam induced generalized urticaria. When CAST® and histamine release assays were applied to the investigation of hypersensitivity to NSAIDs, histamine release assays were said to be less sensitive and correlations poor. With regard to skin tests, positive CAST® results have been detected in some skin test negative cases and where no skin tests exist, for example, hypersensitivity to NSAIDs and non-IgE-mediated drug allergies, CAST® has been advanced as a valuable in vitro alternative approach to diagnosis.

When CAST® results are compared to allergen-specific serum IgE antibody results, correlations are found but they are generally not high, for example, for protein allergens involved in inhalant allergies. However, with allergy to β-lactam antibiotics, the determination of drug-specific IgE is claimed to be less sensitive than the CAST®.

In one early study on 25 patients with hypersensitivity to NSAIDs, the CAST® assay was positive in 5 of 8 aspirin-intolerant and 8 of 12 diclofenac-intolerant patients. Sensitivity of the test was in the range 62.5–80 % and specificity 70–100 % leading the investigators to conclude that the CAST® assay might be a useful in vitro test to reliably and safely screen for hypersensitive reactions to NSAIDs. A comparative evaluation of the CAST® and histamine release assays on 55 patients with immediate reactions to drugs (30 to β-lactams, 19 to aspirin, and 6 to acetaminophen) and 64 nonallergic but drug-exposed controls showed that the CAST® gave slightly better results with only 19 of 55 (13 for β-lactams, 4 for aspirin, 2 for acetaminophen) and 9 of 64 positive while histamine release exceeded 5 % in 28 and 34 cases. Sensitivities (percents) for the CAST® and histamine tests, respectively, were: for β-lactams 43 and 53; for aspirin 21 and 53; for acetaminophen 33 and 33. Corresponding specificities (percents) were 79 and 55; 88 and 35; 100 and 43. The efficiency of both tests was low and the conclusion reached was that the test for released cysteinyl leukotrienes showed little or no diagnostic utility and was not any better than the histamine release assay when applied to the three drugs. Results from a European multicenter study that evaluated the efficiencies of skin tests, serum IgE antibody tests, and CAST® assays in the diagnosis of β-lactam allergy have been used to claim that the CAST® assays "are a decisive and powerful in vitro tool for clear diagnosis of β-lactam allergies ahead of sIgE." Sensitivities and specificities of the three different tests were (percentages): Skin tests, 70 and 100; serum IgE antibodies, 30 and 86; CAST® ELISA/Flow CAST®, 41 and 86. Attention is drawn here to the relatively low figures for IgE antibody tests, especially the low sensitivity result. It can be argued that the currently employed assays for individual penicillins and cephalosporins are inadequate to detect the range of allergenic specificities found in the sera of β-lactam-allergic patients. This is especially true for the minor determinants of the penicillins and R1 and R2 side chain determinants of cephalosporins. This subject is discussed in detail in Chap. 5.

In summing up, it seems true to say that overall, the efficiency of the CAST® tests still falls short for the confident diagnosis of drug allergies. Further application of the tests to drugs apart from the β-lactams and NSAIDs will reveal whether or not the tests become part of the standard diagnostic protocol for investigating drug allergies.

4.6 Basophil Activation Test

4.6.1 Basophils and Background to the Basophil Activation Test (BAT)

Basophils are granulocytes that develop from CD34+ progenitor stem cells in the bone marrow. They comprise less than 1 % of nucleated blood

cells in humans and remain one of the least understood leukocytes. Together with mast cells, they express the high affinity immunoglobulin E receptor FcεRI and have cytoplasmic granules containing preformed histamine. Basophils also express a variety of other receptors including those for complement, interleukins, chemokines, and prostaglandins. Intermittent efforts to utilize the basophil for the development of an in vitro allergy diagnostic test have occurred over many years. Approaches have usually centered on challenge with allergen followed by the attempted measurement of basophil mediators such as histamine and cysteinyl leukotrienes or direct microscopic observation of basophil degranulation. For a number of reasons including the small number of basophils in blood, difficulties involved in handling them, and the laborious and time-consuming nature of the required microscopic counting, these approaches never became widely accepted and used. The development of flow cytometry offered the prospect of working with larger numbers of cells but, initially, advances were limited by the inability to specifically identify basophils among the whole leukocyte population. With improvements in flow cytometric technology, the utilization of reliable membrane markers for basophil identification and activation and the availability of monoclonal antibodies specific for the range of important cell receptors, the monitoring of basophil activation upon allergen challenge became a standardized tool for in vitro diagnosis of immediate hypersensitivity and for recognition of some pseudo-allergies without IgE involvement.

4.6.2 Basophil Activation Markers

The identification of basophil cells was initially based on the presence of the high affinity receptor for immunoglobulin E and expression of CD45 or protein tyrosine phosphatase receptor type C (PTPRC) also known as leukocyte common antigen, a PTP signaling molecule found on all leukocytes. To distinguish basophils from other leukocyte populations, a number of selection strategies are possible. One procedure involves employment of a fluorescence-labeled monoclonal antibody to the eotaxin receptor CCR3, cysteine–cysteine chemokine receptor 3, a receptor closely associated with asthma and allergy. CCR3 also occurs on eosinophils but they can be distinguished from basophils by the former's increased side scatter. Basophils can also be identified as anti-IgE and CD203c (see below) positive cells. An important advancement was the observation that basophil degranulation was accompanied by the upregulation of lysosomal-associated membrane glycoprotein-3 (LAMP-3; also known as granulophysin), belonging to the tetraspanin (TM4SF) family. Now generally known as CD63, this protein is expressed on the surface of degranulated basophils and is the best-validated activation marker used to quantify basophil activation. When FcεRI receptors on basophils are indirectly cross-linked during allergen interaction with receptor-bound IgE molecules, mediators of hypersensitivity including histamine are released and activation markers such as CD63 are expressed on the cell surface. In resting basophils of both normal and allergic subjects, CD63 is located in the intracellular granules with little surface expression but upon upregulation during exocytosis involving fusion between granules and membrane, CD63 is expressed on the membrane surface in high density. Expression of CD63 on basophils has produced convincing and specific results with some common inhalant and venom allergens but with respect to drugs, some early studies reported sensitivities of only 50–64 %, that is, not sufficient to be clinically useful. It was suggested that a contributing factor to this poor sensitivity may be the expression of CD63 on other activated leukocytes, including platelets, and the subsequent adhesion of these other cells to basophils. A more specific and sensitive activation marker therefore seemed desirable. In 1999 the monoclonal antibody 97A6 defined a novel surface antigen belonging to the type II transmembrane protein family on human basophils. The antigen, ectonucleotide pyrophosphatase phosphodiesterase 3 (E-NPPS3), referred to as CD203c, catalyzes the cleavage of oligonucleotides, nucleoside phosphates, and NAD. CD203c is constitutively

expressed on basophils and has the desirable feature of being expressed apparently on that cell alone. After stimulation with allergen, CD203c is rapidly upregulated making it a valuable marker for basophil activation and hence allergy diagnosis. Interestingly, it was thought that the release of histamine is not directly associated with expression of CD63 and CD203c but recently, CD63 expression has been shown to result from only the anaphylactic degranulation form of histamine release.

In a comparison of the performances of CD63 and CD203c in the diagnosis of latex allergy, the sensitivities of the two markers were 50 and 75 %, respectively. Following allergen challenge, levels of expressed CD203c were increased up to 350 % above control values whereas the increase for CD63 was less than 100 %. Expressed as a percentage of basophils that were marker-positive, the result for CD203c was 48 % and for CD63 below 20 %. This led to a clear distinction between resting and activated basophils. Another stated advantage of CD203c was said to be a three- to eightfold higher fluorescence signal than CD63 but others have reported the opposite finding. Occasional weak spontaneous expression of CD203c on resting basophils can make cell identification difficult but rapid expression of the marker following allergen challenge may allow for single color testing without additional staining. A small number of comparative studies have revealed some other clear differences in the activation of CD63 and CD203c. Upregulation of CD203c seems to occur in all or most basophils while upregulation of CD63 produces one population of basophils expressing the marker with high intensity and another population with lower CD63 expression. Expression of CD203c is influenced by some differences in enzymic regulation, activation by prostaglandin D_2 or IL-3 are different and CD203c is more easily activated nonspecifically by, for example, handling of blood and experimental manipulations as well as clinical conditions such as atopic dermatitis and food allergy.

Overall, the claimed superior performance of CD203c has been questioned in more than one study with comments that the presently widely used basophil activation monitored by expression of CD63 is a validated test while the more recently introduced marker requires more extensive study and validation for different clinical conditions. Some have claimed that CD203c produces slightly improved sensitivity if not by itself then together with CD63. The use of both markers has been advocated, and the practice of using dual markers now seems to be common. More recently identified basophil activation markers like CD13, CD107a, and CD164 may be the forerunners of a second generation of BATs.

Recently, it has been shown that phosphorylation of p38 MAPK accompanies upregulation of CD63 expression offering the prospect that measurement of phosphorylation of these mitogen-activated protein kinases might be another way of measuring basophil activation when applying the test to the diagnosis of allergy.

4.6.3 Some Technical Aspects

Ideally, cells should be used in the test within 3 h of blood sampling. Heparin, EDTA, or acid-citrate-dextrose can be used to prevent clotting and blood in the latter two media but not in heparin can be stored for 24 h at 4 °C if necessary. At room temperature, desorption of IgE is thought to occur. Use of whole, heparinized blood is often preferred since it is simple and practical, involving fewer preparative steps while at the same time preserving the basophils' natural environment. Isolated (or more accurately enriched) leukocytes can be prepared as a buffy coat fraction (centrifugation at 500 g for 10 min) or as a leukocyte fraction prepared on a simple density gradient. Isolated leukocytes are more difficult to standardize than whole blood samples, and the conditions of their isolation may affect their reactivity for example, when used with NSAIDs. Some reports show that isolated leukocytes are less sensitive to anti-IgE and allergen-induced CD63 activation, and although this certainly appears to be the case for most drugs, some results with the neuromuscular blocker rocuronium revealed a sensitivity of 92 %. For drug-induced IgE antibody-mediated activation of basophils, parenteral preparations of

drugs are preferred for challenge. Prewarming of blood/cells and reagents at 37 °C is recommended. Activation usually begins within 3 min and reaches a peak within 15–20 min, but this may vary with the marker. Expression of CD203c peaks within a few minutes and begins to decline after 15–20 min. CD203c and CD63 both disappear after 4–5 h. IL-3 has been reported to increase CD63 expression and thus sensitivity. Since drugs are sometimes poor stimulators of expression, addition of IL-3 has been suggested but the enhancing effect of IL-3 does not seem to be consistent.

In theory, utilization of passively sensitization basophils should be possible in basophil activation analyses. This involves stripping of bound IgE antibodies from their receptors on the surface of basophils with the aid of acidic buffers (often a lactic acid buffer, pH 3.9), incubation of these stripped cells with patient's serum (containing IgE antibodies to the allergen being investigated) for 1 h at 37 °C and then challenging the passively sensitized basophils with the allergen at the first stage of the BAT. Donor cells for sensitizing should be from a healthy subject whose basophils are known to be good responders. Both unstripped and stripped donor basophils should be included in the controls. This procedure, apart from being laborious with a number of extra steps, carries the risk of nonspecific stimulation or damage to basophils and is difficult to standardize. Results so far indicate rather poor sensitivity, but some researchers who use the method claim good results.

4.6.4 Controls

Incubation of cells with the stimulation buffer provides the all-important negative control, that is, the spontaneous expression of the activation marker. In general, negative controls remain below 5 % in 80 % of cases. For the positive control, anti-IgE, either as a monoclonal or polyclonal antibody, is employed although the latter is generally superior since monoclonal anti-IgE antibodies are often poor activators of basophils. A monoclonal antibody to the high affinity IgE receptor FcεRI can be used as an alternative and more sensitive positive control. This increases activation and the number of responders. A proportion of individuals (about 5–10 %) have cells that are so-called nonresponders, that is, upregulation of activation markers does not follow IgE cross-linkage or FcεRI activation. Apart from nonresponders, false negatives may arise because of technical manipulation problems, poor storage, or too long an interval between the patient's allergic experience and testing (recommended interval 6–12 months). To be able to interpret the BAT, both negative and positive controls need to conform to normal, expected patterns—high backgrounds and false negative responders preclude normal interpretation and any meaningful findings.

4.6.5 Evaluation of Results

In evaluating results of BATs, one needs to take account of the absolute number of basophils evaluated (more than 150 is desirable) and the percentage of activated basophils. Due to the differences in the upregulation of CD63 and CD203c (see above), results for CD63 expression are generally expressed as a percentage of CD63 basophils whereas results for CD203c tend to be expressed as stimulation indices (SI) of the mean fluorescence intensity. Because of the usually small number of patients, arbitrary cutoff points are usually selected as thresholds for positivity. Cutoffs for some drugs that provide the highest sensitivity and specificity determined by ROC curves are: β-Lactams > 5 % with a SI > 2; aspirin and NSAIDs > 5 % and SI > 2; metamizol > 5 % and SI > 5. However, for drugs, where smaller numbers of basophils are usually activated, the calculation of drug-specific thresholds is an absolute requirement and this demands the study of large groups of well-defined patients. For the determination of correct sensitivity, specificity, and predictive values, the adoption of optimal drug-specific positivity thresholds to replace arbitrarily chosen decision thresholds is advocated. In establishing the cutoff points, ROC curves are necessary to establish optimal sensitivity and specificity.

4.6.6 Application to Drug Allergies

BAT is still mainly employed in research settings, but clinical applications are steadily growing. In cases where the skin test is negative and alternative tests are either not applicable (e.g., a serum IgE test for the solvent Cremophor) or unavailable, and/or when the clinician is faced with reliance on a potentially dangerous provocation test, the BAT is increasingly being used in the diagnosis of drug hypersensitivities and to assess safe alternative treatment regimens. Given the technical improvements and advances made with the test, the increasing acquisition of flow cytometric quantification equipment by laboratories, the test's validation for a number of drugs, and, in some cases, the potential of the test to detect non-IgE-mediated basophil activation, it can readily be appreciated why the method continues to be applied to an increasingly wide range of drugs and other therapeutic agents. This list includes the β-lactam antibiotics penicillins, cephalosporins and clavulanic acid, quinolones, neuromuscular blocking drugs, NSAIDs, radiocontrast media, chlorhexidine, omeprazole, methylprednisolone, valacyclovir, some plasma expanders, starch colloids, carboxymethylcellulose, some heparins, patent blue, platinum salts, hyaluronidase, and recombinant hepatitis B vaccine.

For discussions of the application of BAT to individual drugs or groups of drugs, the reader is referred to the following sections: Penicillins, Sect. 5.1.6.3; clavulanic acid, Sect. 5.5; quinolones, Sect. 6.2.3.6; neuromuscular blocking drugs, Sect. 7.4.3.5; hydroxyethyl starch, Sect. 7.6.1; gelatin, Sect. 7.6.2; NSAIDs, Sects. 9.5.2.1.3, 9.5.2.2.3, and Sect. 9.5.4; contrast media, Sect. 10.5.2.3.

4.6.7 Analysis by Flow Cytometry of Intracellular Histamine and Its Release by Activated Basophils at the Single Cell Level

The release of histamine is thought to initially proceed by piecemeal degranulation before conversion to what has been termed anaphylactic degranulation. It has been proposed that expression of the basophil activation marker CD63 is associated with the anaphylactic degranulation form of histamine release whereas CD203c expression is known not to reflect histamine release. Recently, in an attempt to develop a flow cytometric technique to analyze histamine and its release at the single cell level, D. G. Ebo and colleagues in Antwerp studied the expression of the activation markers CD63 and CD203c and employed the histaminase, diamine oxidase (with a fluorochrome label), after application of allergen, anti-IgE, fMLP, phorbol 12-myristate 13-acetate (PMA), ionomycin, and IL-3 to obtain different degranulation profiles. Nineteen birch pollen-allergic patients, five healthy controls, and the recombinant birch pollen allergen Bet v 1 were used in the study. Upon stimulation with allergen, anti-IgE, or fMLP, basophils that upregulated CD203c generally exhibited a bimodal distribution of CD63 expression, but individual cell responses of CD63 expression proved heterogeneous with the detection of low and high CD63 expressing basophilic subpopulations following IgE-mediated stimulation. When activation markers and histamine release at the single basophil level were analyzed, like CD63 expression, diamine oxidase labeling was found to be bimodal. CD203c (bright) CD63 (bright) cells demonstrated clear histamine release while CD203c (bright) CD63 (dim) showed less histamine release. Diamine oxidase labeling of Bet v 1-stimulated cells that did not degranulate was more intense than the labeling seen with cells exposed only to buffer. Expression of CD63 following PMA treatment was poor, supporting an earlier conclusion that phorbol esters do not induce significant anaphylactic degranulation but this did occur following addition of ionomycin to PMA. IL-3 produced an increase of diamine oxidase labeling of primed basophils from some patients.

From the study, one can conclude overall that flow cytometry can be used to examine histamine and its release along with the simultaneous quantification of basophil activation markers. This methodology has been termed "HistaFlow" by the authors. Results demonstrated that the appearance of CD63 indicates anaphylactic degranulation and significant histamine release whereas

expression of CD203c seems to be associated with fusion of small vesicles, piecemeal degranulation, and slow liberation of histamine from activated cells. However, upregulation of CD203c does not per se indicate histamine release, and release can occur without expression of CD63, that is, the expression of both markers can dissociate from histamine release.

The potential of HistaFlow for application to both research and the clinic is apparent. In the former case, the methodology promises to contribute to our understanding of intracellular signaling and degranulation of basophils while flow cytometry's and BAT's already significant contributions to allergy diagnosis are likely to be further advanced. In relation to drug allergy, this is already being demonstrated. Figure 4.9 summarizes findings when flow cytometry and the expression of basophil activation markers were utilized for the analysis of histamine release by individual basophils from a patient who experienced profound hypotension and severe bronchospasm almost immediately after intravenous administration of the cephalosporin cephazolin. Results in this study clearly demonstrated drug-specific IgE antibody-mediated activation of the patient's basophils together with the visually clear-cut demonstration of histamine release by the basophils.

4.6.8 Future Research and Conclusions

Although the BAT remains largely research based, awareness of its relevance to the clinic is growing, and this is reflected in the allergy literature where diagnostic applications are steadily increasing. An attraction of BAT comes from its obvious features that form part of the in vivo response to allergenic challenge and the resultant release of mediators. Basophils do not need to be separated or purified in potentially cell-damaging manipulations and can be examined in their normal milieu together with other cells to achieve reliable results. At present, however, the procedures shortcomings, particularly its sensitivity and diagnostic accuracy, need to be kept in mind but research progress on extra- and intracellular activation markers and a better understanding of relationships between the expression of these markers and the release of mediators promises to significantly improve diagnostic performance. In particular, application of the HistaFlow methodology may lead to an understanding of the mechanisms underlying intracellular signaling and drug- and other agent-induced degranulation of basophils. An area where BAT will continue to be applied is in the examination of allergens, both pure and in crude form, allergoids, vaccines, newly introduced drugs, additives in many forms of medication, recombinant preparations, and drugs and agents where other tests are either not suitable or not available. Passive sensitization of basophils and the subsequent use of the sensitized cells in BAT examinations is one area where greater research effort seems necessary. Being able to routinely employ serum samples, sometimes taken years before, together with cells known to be good responders and consistently obtain reliable results would be a significant advance. Finally, as more knowledge is accumulated of basophil markers and other routes of activation, BAT might prove to be an appropriate and valuable procedure for the study and diagnosis of some IgE-independent drug reactions.

4.7 Tests for Delayed Type Drug Hypersensitivity Reactions

The mechanisms underlying most delayed or non-immediate hypersensitivity drug reactions appear to be heterogeneous and not yet fully understood. The involvement of T cells in delayed type drug hypersensitivity reactions is well established with different subsets of cells implicated. Type IV cell-mediated mechanisms seem to be involved in reactions such as maculopapular rashes and a number of other skin manifestations including bullous and pustular exanthemas and eczema and for the first three of these conditions T cells have been implicated. For the diagnosis of delayed reactions to drugs, predominately cutaneous reactions, delayed reading of intradermal testing and patch testing are sometimes used, but

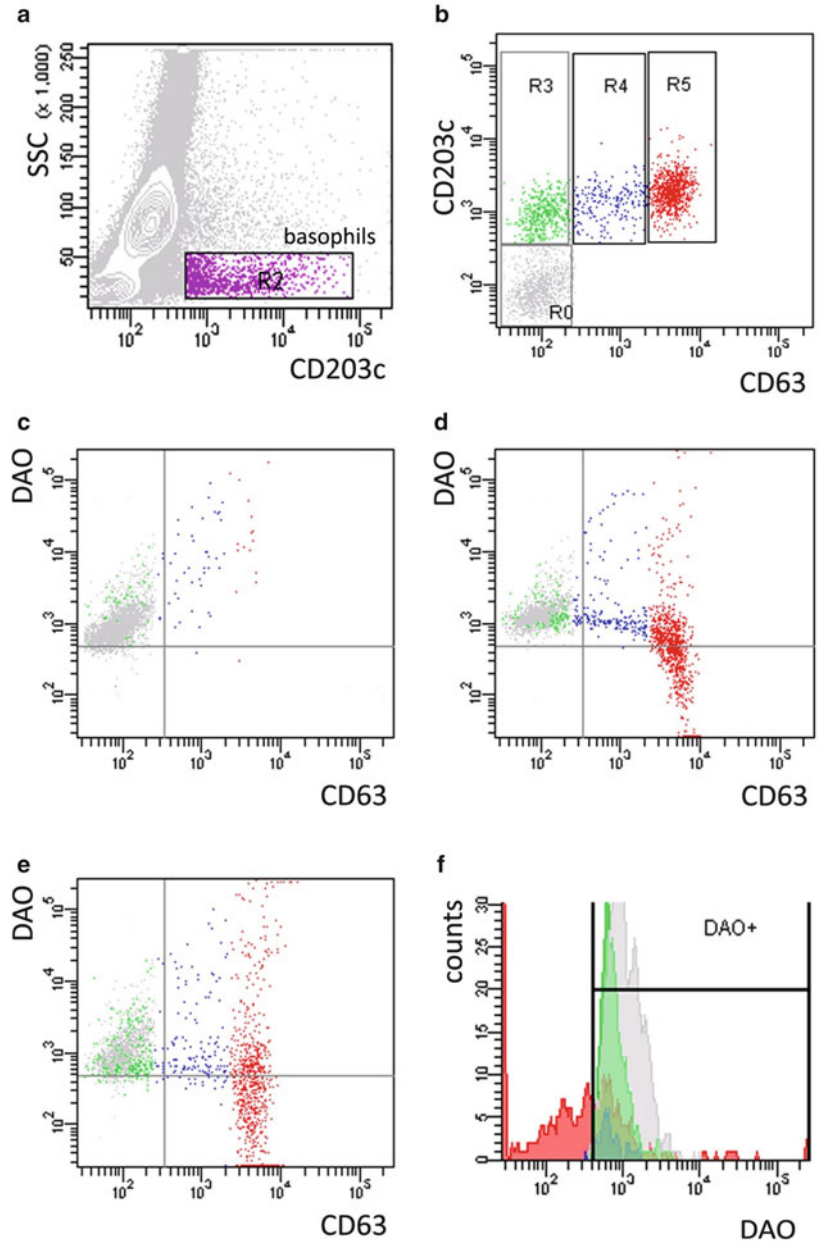

Fig. 4.9 Analysis by flow cytometry of intracellular histamine and its release by activated basophils at the single cell level (Histaflow). Upregulation of basophil activation markers CD63 and CD203c combined with demonstration of intracellular histamine after 20-min activation with cephazolin sodium (Cefacidal®) in a patient with profound hypotension and severe bronchospasm almost immediately after IV administration of cephazolin. Basophils are characterized using side scatter (SSC) and CD203c^{dim+} (*purple population* in R2) (**a**). Activated basophils are defined as anti-IgE and upregulated CD203c$^{bright+}$ positive cells. Co-expression of CD203c$^{bright+}$ and CD63 reveals three subpopulations of distinct CD63 expression; i.e., CD203c$^{bright+}$ CD63^{-} (gate R3, *green*), CD203c$^{bright+}$ CD63^{dim+} (gate R4, *blue*), and CD203c$^{bright+}$ CD63$^{bright+}$ (gate R5, *red*). Gate R0 denotes non-activated CD203^{dim+} basophils (**b**). Histamine-containing basophils are defined as DAO^{+} cells (**c**–**f**). Stimulation with buffer only (negative control) is shown in (**c**). With the positive control stimulated with anti-IgE (**d**), 24 % of the basophils showed histamine release (DAO^{-} cells—see *lower-right quadrant*). Upon stimulation with cephazolin (**e**), 30 % of the basophils demonstrated histamine release, (DAO^{-} cells – see lower right quadrant). (**f**). Shows the DAO histogram (*y*-axis in (**e**)) (Kindly provided by DG Ebo, University Hospital Antwerp. See Ebo DG et al. Analyzing histamine release by flow cytometry (HistaFlow): a novel instrument to study the degranulation patterns of basophils. J Immunol Methods. 2012;375:30)

these skin tests often give negative results even in patients with a clear history of delayed hypersensitivity. The lymphocyte transformation test is often claimed to be the only test to detect drug-sensitized T cells. It can sometimes identify the culprit drug and it begins our list of tests for delayed drug reactions.

4.7.1 Lymphocyte Transformation Test

The lymphocyte transformation test is an in vitro procedure that measures the antigen-induced proliferation of drug-specific T cells. Briefly, peripheral blood mononuclear cells are isolated with a Ficoll gradient and cultured with the addition of autologous plasma in triplicate for 5–7 days in the presence of different concentrations of the drug being investigated. As positive and negative controls, cells in triplicate are incubated with and without phytohemagglutinin 5 μg/ml respectively. Twenty-four hours before harvesting, ^{3}H-thymidine 1 μC is added. Proliferation of cells reflected in the incorporation of radioactive nucleoside into DNA is measured in a liquid scintillation counter and the results expressed as a stimulation index SI where SI equals the counts per minute (cpm) with drug divided by cpm without drug. Some investigators on the basis of their experience with the method regard a SI of 1.8 as positive while a cutoff of 2 is also often used with a value between two and three regarded as a weak positive. Instead of using a radiolabeled nucleoside for incorporation, fluorochrome-labeled derivatives of deoxyuridine (with or without nucleoside-specific antibodies) are sometimes used.

There are a number of practical issues that act against the routine and widespread application of the lymphocyte activation test for the diagnosis of drug hypersensitivities. In the first instance, the test does not easily transfer from the laboratory to the clinical situation; the time from setting up the test to recording results is at least 5–7 days; it involves sterile cell culture; the optimum times for testing different drug-induced reactions are not always known; the patient's existing drug therapy may interfere with the test; and the test itself is cumbersome and technically demanding. Perhaps most importantly of all, a positive lymphocyte transformation test does not necessarily reflect the exclusive involvement of T cells. For example, B cells present in PBMC may also proliferate in response to drug challenge. Nevertheless, the test has some features in its favor. These include: The procedures are carried out in vitro so the test is not harmful to the patient nor is there a risk of the patient developing additional drug allergies; new test reagents are not needed for each of the different drugs tested; simultaneous assessments of T cell responses to multiple drugs can be undertaken; positive reactions can be detected to drugs with different pathomechanisms; and the test is claimed to be more sensitive than other tests for drug hypersensitivities. The lymphocyte transfer test measures a memory T cell response and while it can remain positive for as long as 10–20 years after a drug reaction, other patients are found to test negative only 5–8 weeks after onset of a reaction. Ongoing investigations show that the lymphocyte transformation test is a promising method to identify a causative drug in cases of drug eruptions, but it is crucial to perform the test at the right time and that time depends on the type of drug reaction. Findings with maculopapular drug eruptions, SJS, TEN, and DRESS illustrate this. For DRESS, patients should be tested 5–8 weeks after the onset of reactions while for the other three conditions, testing should take place within 1 week of skin rashes. The lymphocyte transformation test is claimed to have a sensitivity of 60–70 %. Specificity is said to be 100 % for carbamazepine and lamotrigine hypersensitivities and 93 % for β-lactam hypersensitivity giving an overall specificity of at least 85 %. It will be interesting to see if these levels of sensitivity are achieved and the high figures for specificity are maintained as more drugs are examined with the test. The most studied drugs in the lymphocyte transformation test are the β-lactam antibiotics and anti-epileptics, particularly carbamazepine. Diseases in which the test has been found to be frequently positive include maculopapular exanthema, bullous exanthema, acute generalized exanthematous

pustulosis, DRESS, and severe anaphylaxis. It is occasionally positive in cases of urticaria, angioedema, and some drug-induced hepatitis and nephritis reactions and rarely positive in fixed drug eruption, vasculitis, and TEN. Positive lymphocyte transformation tests have been found with quite a big range of drugs including β-lactams, macrolides, tetracyclines, sulfonamides, quinolones, antiepileptics, opioids, ACE-inhibitors, anti-tuberculosis drugs, NSAIDs, local anesthetics, pyrazolones, contrast media, neuromuscular blocking drugs, vitamins, and contact allergens such as *p*-phenylenediamine.

Considering the magnitude of the problem of delayed hypersensitivity reactions to drugs and the difficulties associated with the lymphocyte transformation test, there is a need to develop sensitive and specific tests that are more easily and quickly carried out, widely applicable to the many forms of delayed drug reactions and valuable for use in the clinic as well as the research laboratory.

4.7.2 The Local Lymph Node Assay

This formally validated test, based on measuring the proliferative activity of draining lymph node cells from mice following epicutaneous application of the test agent, is now preferred to the guinea pig sensitization test by the FDA, EPA, and OECD as the accepted method for assessing the skin sensitizing potential of chemicals, i.e., for identifying contact allergens. Originally based on measuring the incorporation of radiolabeled thymidine into the DNA of lymph node cells, more recent protocols substitute 5-bromo-2-deoxyuridine in an ELISA or flow cytometric procedure. Although the local lymph node assay is generally applied to assessing the response to sensitizing chemicals used industrially and/or contacted in the environment (e.g., dinitrochlorobenzene, picryl chloride), the method has been applied successfully to detect sensitization by drugs, for example, benzocaine and benzylpenicillin. The method can also be adapted for use as an immune function assay by examining the effect of orally administered drugs on the T cell response provoked by contact sensitizing agents. Confirmation of the results of the assay and extension of their value can be obtained by carrying out concurrent cytokine release measurements.

4.7.3 Toward Nonproliferation-Based In Vitro Assays: Cell Surface Activation Markers, Cytokines, Chemokines, and Skin-Homing Receptors

For the detection of delayed type drug hypersensitivity reactions, the lymphocyte transformation test may be the only readily available and well investigated ex vivo methodology with a sufficiently long-standing pedigree to be employed with any confidence. However, as outlined above, the test has some major limitations including its practicability, lack of specificity to T cells, and the time involved in its execution. Although the BAT more closely mirrors the in vivo pathways leading to allergic manifestations, it cannot be used to detect non-IgE-mediated allergic reactions. With these considerations in mind and with the steady accumulation of insights into cellular and molecular immune processes underlying the secretory and effector functions of antigen-specific T lymphocytes, proliferation-based assays are beginning to be supplemented by some novel in vitro tests to detect and measure cell activation markers, signaling molecules, chemoattractants, transcription factors, and cytolytic molecules released by some lymphocytes. In the main, these investigations are still essentially research based and before any new assay's findings and methodologies can be seriously considered for the diagnosis of drug allergies, studies will have to confirm their usefulness in large numbers of clinically well-defined patients.

4.7.3.1 Cell Surface Activation Markers

Activation markers such as CD69, CD25, CD71, and the MHC class II cell surface receptor HLA-DR are expressed and may be upregulated on the T cell surface. CD69, widely used in vitro and in vivo as a marker of T cell activation for

more than 15 years, was recently exploited in flow cytometry studies for the detection of drug-reactive T cells from patients with delayed type drug hypersensitivities. Freshly isolated PBMC were cultured in the presence of drug or IL-2 for 48 h before examining CD69 expression on CD4+/CD8+ T cells by flow cytometry with fluorescent-labeled monoclonal antibodies to CD69, CD3, CD4, and CD8. Cells from lymphocyte transformation test-positive patients with delayed hypersensitivity showed a significantly increased expression of CD69 following exposure to the culprit drug. Upregulation of the activation marker occurred in 0.5–3 % of T cells with only a minority of the reactive cell population being drug-reactive T cells secreting cytokines. There were a higher number of bystander T cells activated by IL-2 and possibly other cytokines. Although it was concluded that upregulation of CD69 was a promising tool to identify drug-reactive T cells from patients with drug hypersensitivities, developments in this area and progress with this approach have been less than expected.

4.7.3.2 Monitoring of Cytokines from T Cells and the ELISPOT Assay

One relatively new approach is based on the detection of drug-specific cytokine production by cells from patients with a history of drug hypersensitivity following stimulation in vitro with drugs well known to provoke delayed type reactions. During the last decade, work has shown that measurement of secreted cytokines IL-5 and IFN-γ can be useful for diagnosis of drug hypersensitivity. More recently, the secretion of a range of cytokines and chemokines has been examined in attempts to identify promising "markers" for the in vitro detection of T cells sensitized to drugs. As a first approach, PBMC from patients with delayed type drug hypersensitivity were stimulated in vitro with drug for extended periods (generally 72 h), and liberated cytokines were measured in the supernatants using immunoassays for 17 different cytokines/chemokines viz., IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8 (CXCL8), IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, TNF-α, CCL2, and CCL4. Increases in secretion were observed for IL-2, IL-5, IL-13, and IFN-γ in response to sulfonamide and β-lactam drugs in the PBMC from patients allergic to these drugs but no differences were seen in the cytokine secretion patterns of sulfonamide- and β-lactam-reactive PBMC. For PBMC from healthy subjects, sulfonamide and β-lactam drugs stimulated statistically significant increases in IL-1β and IL-6 indicating that before measurement of cytokine/chemokine release can be considered as a likely diagnostic tool, many more patients will need to be examined and more information will be needed on the background spectrum of cytokines secreted in response to different drugs.

In another approach to monitor cytokine release in patients with delayed drug hypersensitivities, flow cytometry and an ELISA assay were used to measure cytokine secretion by PBMC. Drug-induced production of IL-5, a stimulant for B cells and immunoglobulin secretion, the anti-inflammatory cytokine IL-10, and IFN-γ was demonstrated by flow cytometry, and secretion of the T cell growth factor IL-2 was detected in the ELISA assay. Cytokines were not detected in less than a 5-day incubation period. Flow cytometry and ELISA detected drug-specific cytokine production in 75 % and 79 % of patients, respectively. Combining both procedures increased the sensitivity to 100 %. In another study designed to utilize the drug-induced release of cytokines as a sensitive assay for the diagnosis of cutaneous adverse reactions to drugs, detection of IFN-γ was undertaken to investigate T cell involvement in patients with maculopapular exanthema caused by amoxicillin (see below). IFN-γ was selected since it is thought to be important in the pathophysiology of maculopapular exanthema, and the expression of this type 1 cytokine is restricted to activated T cells.

The ELISPOT (enzyme-linked immunospot) assay is based on classical immunoassay principles and the detection procedures employed in ELISA assays. Its sensitivity, ease of use, employment of highly reactive, standardized monoclonal antibodies, commercial availability, and ready application to studies on individual cell types makes it a good choice for research and diagnostic

investigations of cellular effector–target interactions. The methodology involves specific capture and immobilization of the target molecule (e.g., a cytokine) with a complementary monoclonal (usually) antibody and visualization of the reaction by addition of a detecting (second) antibody tagged with a highly sensitive enzyme label, for example, an anti-cytokine labeled with streptavidin-alkaline phosphatase. The ELISPOT assay was employed to monitor the appearance of IFN-γ following amoxicillin and ceftriaxone challenge of the PBMC of 22 patients with a well-documented history of delayed hypersensitivity to β-lactams manifesting as maculopapular exanthema. The assay detected IFN-γ, and hence drug-specific T cells, in 20 of the 22 patients examined providing evidence that maculopapular exanthema is mediated by IFN-γ-producing T cells. Results showed that the ELISPOT assay can detect amoxicillin-specific T cell precursors as low as 1:30,000 blood leukocytes and with a frequency of 30–125 per 10^6 PBMC; that the test can distinguish patients with immediate and delayed reactions; T cells that cross-react with other β-lactams can be detected; and the ELISPOT assay is more sensitive than the lymphocyte transformation test for the diagnosis of delayed type hypersensitivity to β-lactams. The sensitivity and specificity of the assay for the diagnosis of delayed type hypersensitivity to β-lactams were 91 % and 95 % respectively. From the study, the investigators concluded that other Th1 and Th2 cytokine-producing cells should be examined in sensitive assays with the view to detecting specific T cells and improving the diagnosis of many drug-induced delayed reactions and it was suggested that the ELISPOT assay might represent an important advance in the quest for improved tests for in vitro ex vivo diagnosis of such reactions.

4.7.3.3 Granzyme B ELISPOT Assay for the Detection of Drug-Reactive T Cells

An interesting and highly promising candidate marker protein for drug-specific T cells is the serine protease granzyme B expressed by cytotoxic T lymphocytes and natural killer (NK) cells. Granzyme B is constitutively expressed by memory but not by naïve cytotoxic T lymphocytes making the protease a likely candidate to be utilized for the assessment of cell-mediated cytotoxicity. A highly sensitive ELISPOT assay has been developed for granzyme B but, unlike the IFN-γ ELISPOT assay, it directly measures the release of a cytolytic protein making it a more direct measure of antigen-specific cytotoxic T lymphocyte lytic activity. An additional advantage of monitoring granzyme B secretion is its early release following effector–target contact. The enzyme is detectable as early as 10 min after interaction, significant amounts are measurable after 30 min, and maximum levels are obtained after 4 h. Measurable amounts of INF-γ are seen only after 1 h. An ELISPOT assay for granzyme B and surface expression of CD107a were recently utilized for the detection of cytotoxic and NK cells in peripheral blood of patients with various drug-induced skin diseases. CD107a, also known as LAMP-1 (lysosomal-associated membrane protein 1), is a membrane glycoprotein degranulation marker on CD8+ lymphocytes and NK cells. The assay proved highly specific for detecting drug-reactive cytotoxic cells in peripheral blood of drug-allergic patients but no strict correlation between the granzyme B assay and the lymphocyte transformation test was found. Both CD4+ and CD8+ T cells expressed CD107a supporting belief both of their involvement in drug hypersensitivities and the contribution of NK cells to the observed drug-induced degranulation.

4.7.3.4 Chemokines and Skin-Homing of T Cells

The skin-associated chemokine CCL27, also called Skinkine, Eskine, and CTACK (cutaneous T cell-attracting chemokine) and its receptor CCR10 are associated with skin-homing of T lymphocytes and are implicated in T cell-mediated inflammation of the skin. Most skin-infiltrating lymphocytes in patients suffering from contact dermatitis and psoriasis express CCR10 and CCL27–CCR10 interactions appear

to play an important role in T cell-mediated skin inflammation. CCL27 can be induced by TNF and IL-1β and suppressed by glucocorticosteroids. Intracutaneous injection of CCL27 attracts lymphocytes and neutralization of the interaction of the chemokine with its receptor impairs lymphocyte recruitment to the skin and suppresses allergen-induced inflammation. Utilization of this knowledge of the CCL27–CCR10 interaction might provide the basis of investigations that lead to a better understanding of T cell-mediated skin inflammation in different delayed type drug hypersensitivity reactions and stimulation of further investigations of other chemokine–receptor interactions of skin-infiltrating lymphocytes. Some encouraging in vitro test results with the CCL27-CCR10 interaction are beginning to appear, for example, results demonstrating that levels of expressed CCL27 in skin biopsies from two patients with bullous skin reactions were higher than those found in healthy subjects and other drug-induced exanthemas, and resolution was associated with return to normal expression levels of both CCL27 and its receptor. These findings indicate that the CCL27–CCR10 interaction may be involved in the selective recruitment to the skin of certain cytotoxic lymphocytes in SJS and TEN and this and other chemokine-T cell receptor interactions may be involved in other drug-induced cutaneous reactions.

4.7.3.5 Cutaneous Lymphocyte-Associated Antigen

Cutaneous lymphocyte-associated antigen (CLA) is a L-fucose-containing carbohydrate epitope on the P-selectin glycoprotein ligand-1, a surface glycoprotein expressed on the majority of peripheral blood leukocytes. Most T cells that infiltrate the skin express CLA, and CLA positive cells have been implicated in contact dermatitis to nickel in some patients with delayed cutaneous allergic reactions. Studies on the expression of CLA by T cells from patients with exanthematous reactions induced by a range of drugs including β-lactams have so far shown that CLA positive cells appear to parallel the evolution of the disease and they may be involved in the underlying pathophysiologic mechanisms. Patient numbers were small, and although CLA must be regarded as a promising marker for aiding efforts to understand the relationship between T cells, drugs, and adverse delayed skin reactions, research in this area must be seen as being still in its infancy.

4.8 And Finally: Is the Patient Allergic to the Drug?

The aim must be to establish or disprove a causal relationship between the drug and the patient's reaction and, if there is such a relationship, the reaction type and mechanisms should be determined if possible. The approach should be both methodical and meticulous. For a reaction that is severe or life-threatening, the drug should not be readministered. If the reaction is a type A adverse drug reaction due to the drug's pharmacological effects (see Sect. 1.1.2), lowering the dose may be all that is necessary to avoid, for example, a toxic reaction or known side effect and for medication to safely continue. For a reaction that is not severe, a challenge test to confirm or eliminate suspicion can be undertaken. For type B immunologic reactions it is necessary to establish the underlying mechanism. This can be done by employing confirmatory tests if they are available. In many cases, if not most, such tests are not available, and then it may be necessary to avoid the drug as a precaution and prescribe an alternative drug if one is suitable and available. Otherwise, a graded challenge with the implicated drug can be carried out, but this should be done only if the reaction was not life-threatening and clearly not an IgE-antibody-mediated reaction. Nevertheless, if the medication is essential or highly desirable, appropriate desensitization (Sect. 3.5) should be considered. The overall strategy in looking for a causal relationship and the methodical approach pursued is summarized in Fig. 4.10.

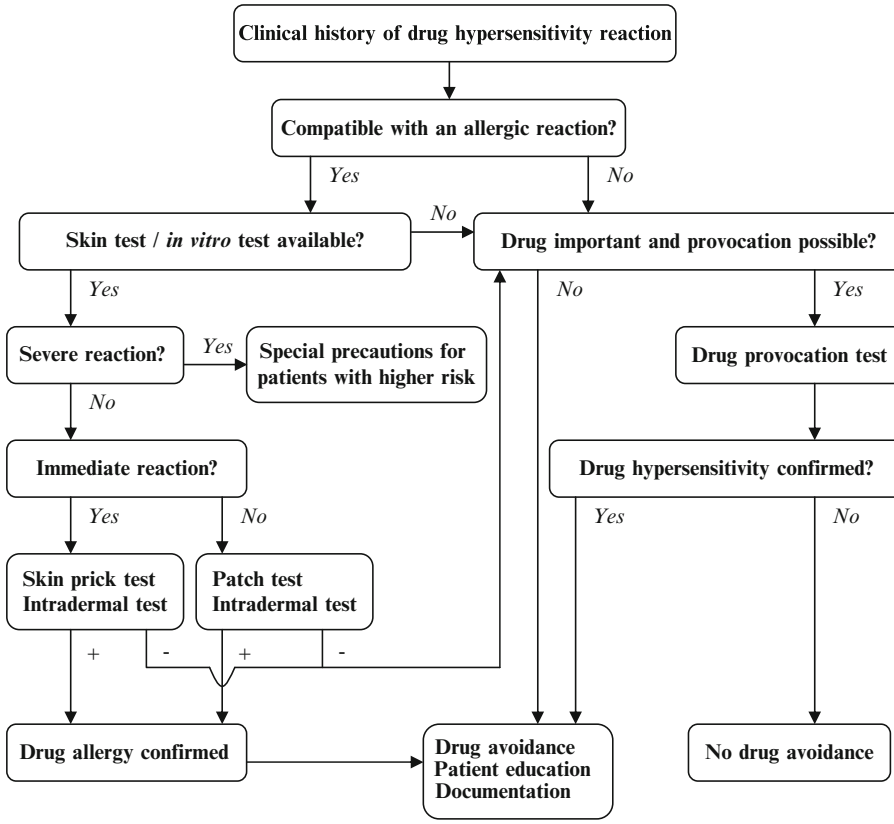

Fig. 4.10 Algorithm for the use of skin testing (and suitable available in vitro tests) in the diagnosis of drug hypersensitivities. From Brockow K et al. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. Allergy 2002;57:45. Reproduced with permission from John Wiley and Sons

Summary

- In diagnosing drug allergies, the patient's history, skin testing, some in vitro laboratory tests, and the challenge test are the backbone of the investigative procedures.
- A detailed and thorough clinical history of the patient is the most important component of the diagnostic process.
- If skin prick testing elicits no reaction, intradermal testing is usually employed. The latter test is more sensitive but produces more false positives, that is, it is less specific.
- Depending on the drug, a prick test wheal diameter at least 3 mm greater than the negative (saline) control or a wheal at least half the diameter of the positive (histamine) control is considered a positive result.
- Depending on the drug and the severity of the patient's drug reaction, the initial intradermal test injection may range from a small dilution of 1:10 or 1:100 of the prick test concentration to more extreme dilutions of up to 1:100,000. If no reaction is seen, the concentration is increased in logarithmic steps until the final concentration is reached, and this maximum concentration should not be exceeded. A positive reaction is an increase in diameter of more than 3mm over the initial 20 ul injection bleb (usually ~2 mm) accompanied by erythema.
- Intradermal testing is normally contraindicated in patients who have developed SJS, TEN, and erythema multiforme.
- Skin tests should be read at 15–20 min for immediate reactions and 48–72 h (or sometimes later) for delayed reactions.

- The patch test is both a screening test for hypersensitivity and a provocation test in the target organ, the skin.
- If pure drug is used for patch testing, concentrations should begin at about 0.1 % and progress to 1–10 % if results are negative. For DRESS, SJS and TEN (if testing *must* be done) and for some drugs, for example, aciclovir, carbamazepine, and pseudoephedrine, testing should start with lower concentrations.
- Patch test reactions are read after 48 and 72 or 96 h. In some cases a reaction occurs in less than 2 days (e.g., abacavir) while with some other drugs such as corticosteroids, aminoglycoside antibiotics, and phenylephrine, reactions may occur after 6 or 7 days.
- The COADEX classification should be used to assess clinical relevance of positive patch tests.
- Assays for drug-specific serum IgE antibodies are useful in cases of skin test-negative or equivocal reactors or when skin tests are unreliable or unavailable.
- In interpreting results of IgE antibody tests, ROC curves provide more information to aid discrimination between positive and negative results. The concentration of IgE antibodies assessed by immunoassay is related to the presence of allergic symptoms.
- A drug challenge, or provocation test, is the controlled step-wise administration of a drug in a supervised hospital environment in order to determine if the drug was the causative agent in a patient's allergic reaction. The test is the best way to confirm an allergic reaction, and it is considered to be the "gold standard" in the diagnosis of drug hypersensitivity reactions.
- Drug challenge tests are particularly important when other usually employed tests, particularly skin tests, are not available or possible.
- Increased mast cell tryptase concentrations are a valuable indicator of an anaphylactic reaction especially during anesthesia and although elevated levels favor an IgE-antibody-mediated cause, they do not always distinguish between an anaphylactic and an anaphylactoid reaction. Normal levels of mature tryptase in serum are less than 1 ng/ml whereas levels equal to or above 1 ng/ml indicate mast cell activation. In anaphylaxis, the ratio of total to mature tryptase is typically less than 10.
- Histamine release from human blood leukocytes after challenge with drug in vitro is occasionally employed but in general, for diagnostic purposes, histamine concentrations in biological fluids have rarely been routinely measured; the half-life of histamine in plasma is short—approximately 1–2 min.
- The release of cysteinyl leukotrienes from isolated peripheral blood leukocytes following allergen challenge in vitro has been utilized as a test for immediate hypersensitivity in the form of the Bühlmann CAST® (Cellular Allergy Stimulation Test) assays offered commercially as CAST® ELISA a microtiter plate ELIZA immunoassay and as a flow cytometric assay Flow CAST®. Although showing some promising results with β-lactams and NSAIDs, wider assessment of the methods in more laboratories is needed.
- Given the technical improvements and advances made with BAT, the increasing acquisition of flow cytometric quantification equipment by laboratories, the test's validation for a number of drugs, and, in some cases, the potential of the test to detect non-IgE-mediated basophil activation, it can be appreciated why the method continues to be applied to an increasingly wide range of drugs and other therapeutic agents. In a new application of the methodology termed "Histaflow," flow cytometry has been used to examine histamine and its release along with the simultaneous quantification of basophil activation markers.
- In vitro tests for delayed drug reactions include the lymphocyte transformation test and local lymph node assay but nonproliferation-based in vitro assays of cell surface activation markers, cytokines, chemokines, and skin-homing receptors will be increasingly applied.
- The ELISPOT assay shows potential for use in drug allergy diagnosis. It can detect amoxicillin-specific T cell precursors as low as 1 : 30,000 blood leukocytes and can distinguish patients with immediate and delayed reactions. The assay is more sensitive than the lymphocyte transformation test for the diagnosis of delayed type hypersensitivity to β-lactams.

- A highly sensitive ELISPOT assay has been developed for granzyme B but, unlike the IFN-γ ELISPOT assay, it directly measures the release of a cytolytic protein making it a more direct measure of antigen-specific cytotoxic T lymphocyte lytic activity.
- Studies on chemokines such as CCL27 associated with skin-homing of T cells, and cutaneous lymphocyte-associated antigen CLA, are promising markers for aiding efforts to understand the relationship between T cells, drugs, and adverse delayed skin reactions.

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β-Lactam Antibiotics

5

Abstract

The β-lactam antibiotics comprise four main classes of drugs; penams (penicillins), cephems (cephalosporins), monobactams, and carbapenems. Penicillins can cause all four types of hypersensitivity responses. IgE antibodies in patients' sera detect a spectrum of antigenic specificities, show heterogeneous recognition and cross-reactive responses, and may distinguish fine structural features, e.g., amoxicilloyl and amoxicillanyl determinants. With a negative history of penicillin allergy, the incidence of positive skin tests is 2–7 %. For skin test-positive patients the risk of an acute allergic reaction ranges from 10 % (negative history) to 50–70 % (positive history). IDTs with delayed reading and patch tests are used to diagnose delayed reactions. Aminolysis of cephalosporins produces unstable intermediates that decompose to penaldate and penamaldate structures resulting in only the R1 side chain remaining from the original molecule. With some allergic patients the R2 side chain and/or the whole cephalosporin molecule are also recognized by IgE antibodies. Testing with penicillins does not reliably predict cephalosporin allergy unless the side chains of the penicillin and the culprit cephalosporin are similar. Aztreonam shows little, if any, cross-reaction with penicillins and cephalosporins. The practice of avoiding imipenem and meropenem therapies in penicillin-allergic patients should be reconsidered. There has been an increase in cases of immediate hypersensitivity to clavulanic acid.

So named because of the presence of a four-membered β-lactam ring in the molecules, the β-lactam antibiotics comprise four main classes of drugs that possess antibacterial action, viz., penams (penicillins), cephems (cephalosporins), monobactams, and carbapenems. The β-lactam ring is fused to a thiazolidine ring in penams, a dihydrothiazine ring in cephems, and a dihydropyrrole ring in the carbapenems. Monobactams consist of a β-lactam ring free of any other ring attachment. Other classes of β-lactam antibacterials, each with a small number of less often used drugs, are the penems, clavams, carbacephems, oxacephems, and cephamycins (Fig. 5.1). Henceforth here, penams and cephems are referred to by the more commonly used names,

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_5, © Springer Science+Business Media, LLC 2013

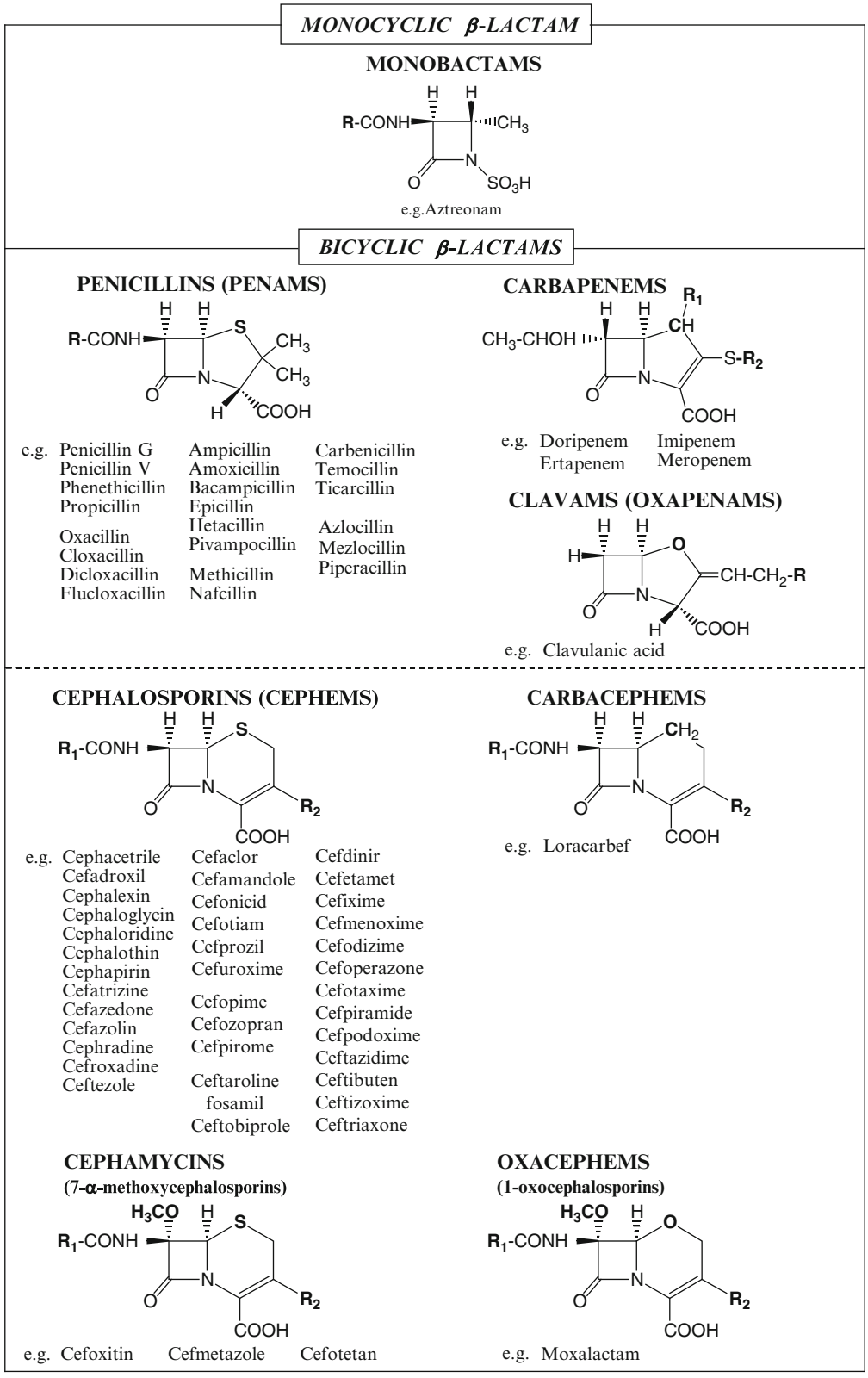


Fig. 5.1 Structures of the four main classes of β-lactam antibiotics, penams (penicillins), cephems (cephalosporins), carbapenems, and monobactams. Less frequently used β-lactam antibacterials include the carbacephems, oxacephems, cephamycins, clavams (see Sect. 5.5 and Fig. 5.18d), and penems (not shown)

penicillins and cephalosporins, respectively. These two drug classes of antibacterials are highly effective in treating infections and generally not toxic and, even after extensive use over many decades and the generation of resistant organisms, penicillins and cephalosporins remain the most frequently prescribed antibiotics. However, soon after the introduction of the first penicillin, the propensity of this group of drugs to cause allergic reactions ranging from simple rashes to life-threatening anaphylaxis was recognized. Penicillins, together with cephalosporins, are probably still the most common cause of drug allergy and this continues to be a problem in antibiotic selection and risk avoidance today.

In this chapter, the four main classes of β-lactams will be dealt with separately with greatest emphasis on the heavily used penicillins and cephalosporins and with reference to the other classes when appropriate. Despite the impressive immunochemical insights from the pioneering drug allergy studies that began over 50 years ago with the early investigations of Levine, Parker, De Weck, and Dewdney, clinically relevant molecular aspects of β-lactam drugs have been largely ignored in recent years with steps necessary to identify and define allergenic structures and improve diagnostic agents neglected. While a full and reliable case history, skin tests, challenge tests, and serum IgE antibody investigations still form the basis of an accurate diagnosis, a good knowledge of different allergenic structures and an improved range of test materials for the recognition of individual sensitivities would assist clinicians in achieving accurate and precise diagnoses, identifying likely cross-reactive drugs and selecting safe alternative drugs.

5.1 Penicillins

5.1.1 Incidence of Penicillin Hypersensitivity and Clinical Aspects

The incidence of hypersensitivity reactions to penicillins is about 1–2 % but, importantly, up to about 10 % of patients taking a penicillin report, or believe, that they are allergic to the medication and 80–90 % of patients who claim to be allergic to penicillins are not. It has been claimed that virtually all patients with a negative skin test to the drug(s) can take penicillins without serious sequelae. Penicillins have long been known to be the most common cause of both drug-induced anaphylaxis and drug-induced allergic reactions causing an estimated 75 % (500–1,000) of deaths each year in the USA and 26 % of fatal drug-induced anaphylaxis in the UK.

Penicillins can cause all four types of hypersensitivity responses provoking type I IgE-mediated reactions such as urticaria, angioedema, asthma, and anaphylaxis; type II antibody-mediated hemolytic anemia and thrombocytopenia; type III immune complex-mediated serum sickness-like reactions and vasculitis; and type IV T cell-mediated contact dermatitis, rashes, and other skin eruptions (refer to Chaps. 2 and 3). Table 5.1 lists clinical adverse reactions, together with their immune

Table 5.1 Clinical adverse reactions to penicillin and associated immune mechanisms

Immediate IgE-mediated reactions (Type I)
Urticaria
Angioedema
Asthma
Anaphylaxis
Urticaria
Angioedema
Laryngeal edema
Flushing
Pruritus
Nausea, vomiting, diarrhea, headache
Bronchospasm
Tachycardia, arrhythmias
Cardiovascular collapse
Non-immediate reactions, not IgE-mediated
Antibody-mediated (Type II, cytotoxic)
Hemolytic anemia
Thrombocytopenia
Immune complex-mediated (Type III)
Vasculitis
Serum sickness
T cell-mediated (Type IV)
Contact dermatitis
Drug-induced skin eruptions

mechanisms, that may occur following the administration of a penicillin. As well as a classification based on immune mechanisms, reactions are often grouped as immediate, accelerated, or late onset, with immediate reactions occurring within 1 h, accelerated reactions occurring between 1 and 72 h, and late reactions after 72 h. Clinical manifestations can occur singly or in combination—for immediate responses the most common reactions are urticaria and angioedema; urticaria and maculopapular rashes occur commonly in accelerated reactions; and erythema multiforme, skin eruptions, hemolytic anemia, and a serum sickness-like reaction are seen as delayed reactions. Delayed onset urticarial or maculopapular rashes are frequently seen especially in children and many are labeled allergic without evidence or even testing. Allergy is overdiagnosed in these patients since skin rashes are rarely reproducible by challenge testing (Fig. 5.2a, b) and viral infections are suspected in many of the penicillin-induced rashes.

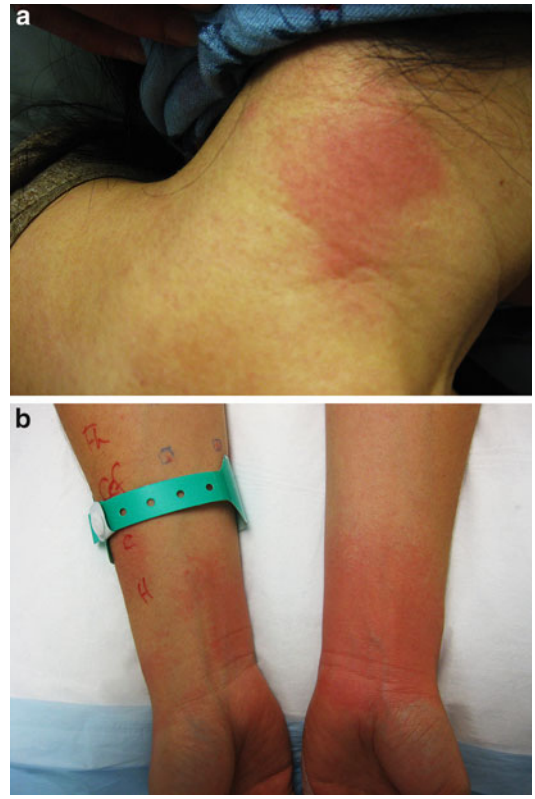


Fig. 5.2 Erythematous rash on a patient's neck **(a)**, arms, and hands **(b)** following oral challenge with amoxicillin. The patient showed a negative intradermal test to the drug and responded on the last oral challenge dose of amoxicillin. (Photographs courtesy of Dr P. A. J. Russo and Dr J. S. Fok, Department of Clinical Immunology and Allergy, Royal Adelaide Hospital, Adelaide)

5.1.2 Penicillin Antigens and Allergenic Determinants

All penicillins contain a β-lactam ring fused to a thiazolidine ring and individual penicillins are distinguished by the nature of the side chain R group (Fig. 5.3). The structures of the most clinically important and frequently used penicillins are shown in Fig. 5.4.

Extensive investigations over a period of more than 30 years on the formation of antigenic and allergenic determinants of benzylpenicillin (penicillin G) led to the unraveling of complex pathways and steps in the formation of a number of ultimately proven, and some putative, allergenic determinants. At the time of that research, the accepted view was that low molecular weight drugs and other chemicals must first combine irreversibly with a macromolecular carrier, usually protein, to produce hapten–carrier complexes that stimulate a specific antibody response. Although this view is still largely accepted, some exceptions may occur (see in particular Chaps. 3 and 7).

5.1.2.1 The Penicilloyl Determinant and Benzylpenicillenic Acid

Of all the penicillin breakdown products and protein conjugates studied, most is known about the penicilloyl determinant. The major populations of antibodies in sera from experimental animals immunized with benzylpenicillin, and from humans following penicillin therapy, were found to be complementary to this determinant leading to its designation as the major penicillin antigen. It was estimated that of all the penicillin molecules that became covalently bound to protein under physiological conditions, 95 % form penicilloyl groups and it was this quantitative predominance rather than allergenic potency or clinical or immunological importance that the

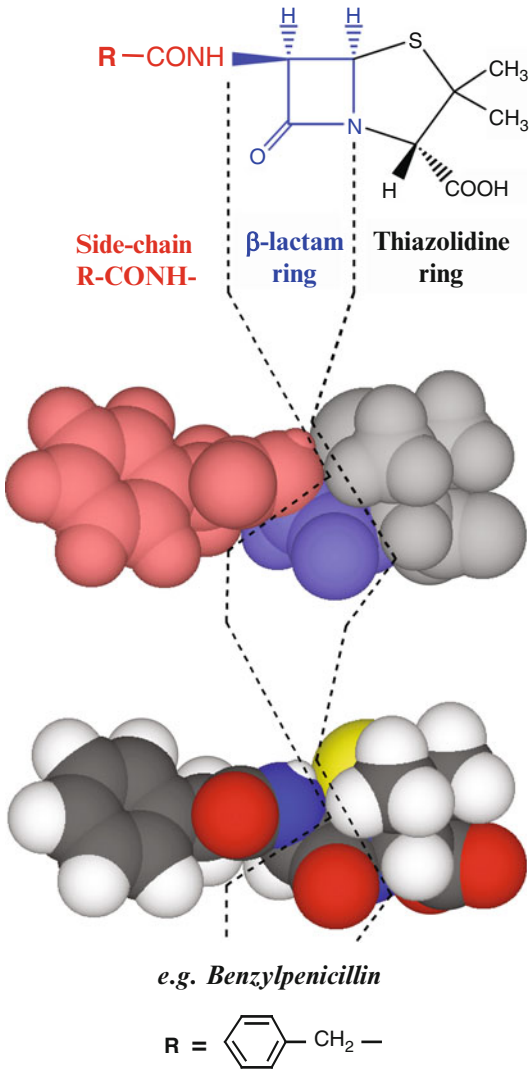


Fig. 5.3 Side-by-side two- and three-dimensional penicillin structure highlighting the side chain (R), β-lactam, and thiazolide ring structures. From Baldo BA. Diagnosis of allergy to penicillins and cephalosporins. Allergy Clin Immunol Int. 2000;12:206. Reprinted with permission from ©2000 Hogrefe & Huber Publishers (now Hogrefe Publishing. http://www.hogrefe.com)

term "major" was applied to. Another potential confusion with the term is the possibility that it may be thought to refer to the major degradative product of the penicillins, viz., penicilloic acid. There is more than one pathway for the formation of the penicilloyl determinant. One is by direct reaction of benzylpenicillin involving the opening of the β-lactam ring and nucleophilic attack on protein amino groups, first demonstrated by Bernard Levine at high pH (Fig. 5.5). Later studies showed that the reaction also proceeds at neutral pH. Benzylpenicillin also readily rearranges to form an isomer, D-benzylpenicillenic acid, a highly reactive compound which, like benzylpenicillin, binds selectively to lysine residues of human serum albumin (HSA) forming penicilloyl–lysine adducts (Fig. 5.5). The rearrangement to penicillenic acid occurs in vitro and in vivo where it is not dependent on enzymatic involvement. A direct demonstration of the presence of penicilloylated protein conjugates in vivo was first achieved by Levine in inhibition experiments with sera from patients treated with high doses of penicillin. Levine argued that formation of the penicilloyl specificity more likely proceeded via the penicillenic intermediate because anti-benzylpenicillin antibodies were specific for a diastereoisomeric mixture of benzylpenicillin, whereas benzylpenicilloyl hapten, formed from benzylpenicillin by direct aminolysis, would produce only the D-α-diastereoisomer. It was later pointed out, however, that epimerization by the direct route is also possible. Recently, the question of diastereoisomeric benzylpenicilloyl antigen formation from benzylpenicillin and benzylpenicillenic acid was investigated in a mass spectrometric and molecular modeling study. Both benzylpenicillin and benzylpenicillenic acid were shown to covalently bind to lysine residues in HSA to form penicilloyl adducts in vitro, but the two compounds showed differences in their binding targets. Whereas the parent drug showed marked preferential binding to Lys199, benzylpenicillenic acid bound to this residue and to Lys525 as well. Characterization of the isomeric adducts, formed when benzylpenicillin and benzylpenicillenic acid were incubated with albumin in vitro, revealed two isomers for both compounds at each of the modified lysines, although the 5R,6R diastereomer predominated for the parent drug (pathway 1, Fig. 5.5) and the 5R,6S diastereomer predominated for the acid (pathway 2, Fig. 5.5). Prolonged incubation of benzylpenicillenic acid with HSA produced an increase in the relative amount of the 5R,6S diastereomer. Investigations showed that epimerization

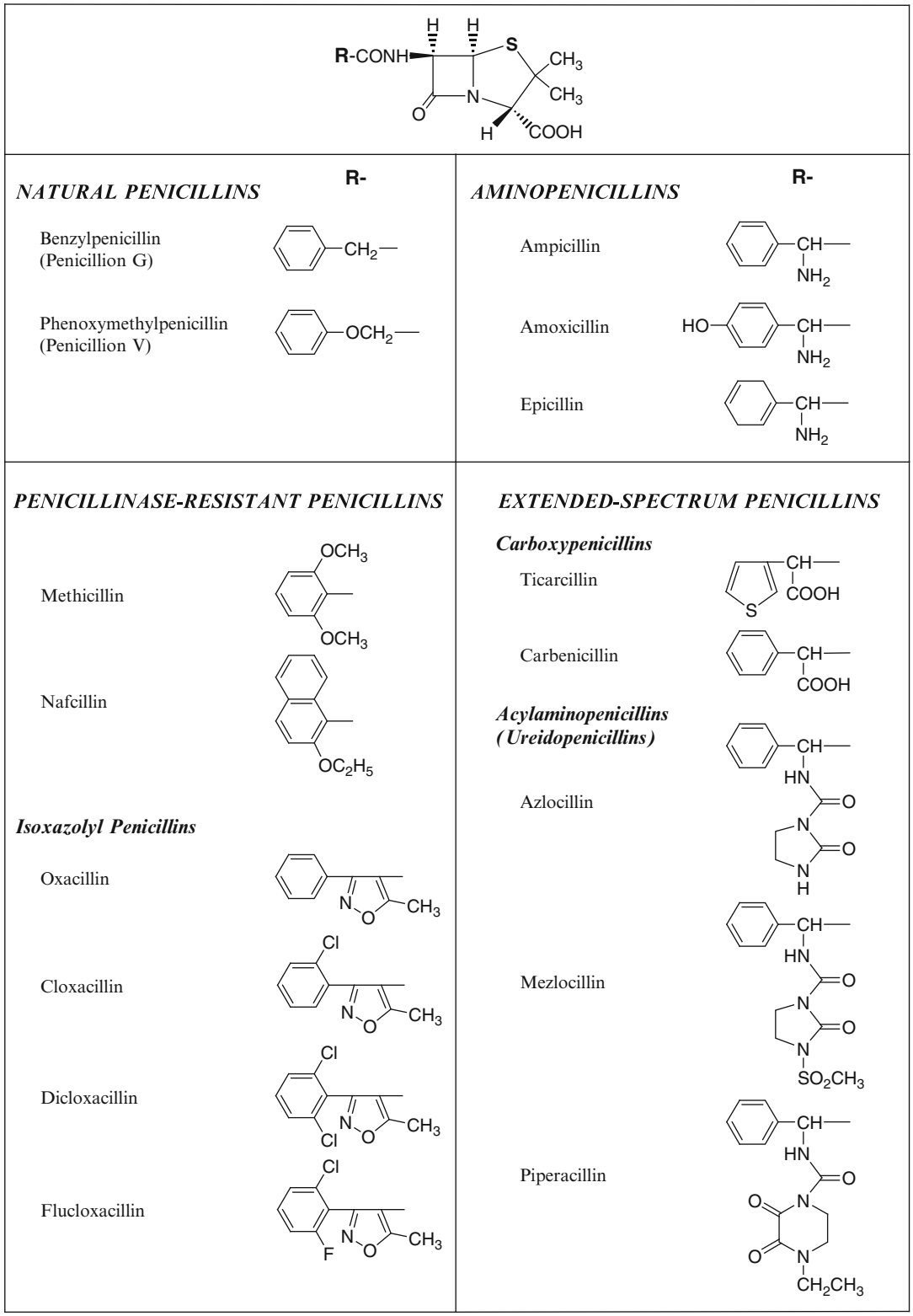


Fig. 5.4 Structures of the different side chain (R) groups on individual penicillins grouped according to their similarities of structure (aminopenicillins, isoxazolylpenicillins, and ureidopenicillins) and biological properties (penicillinase resistance, extended spectrum)

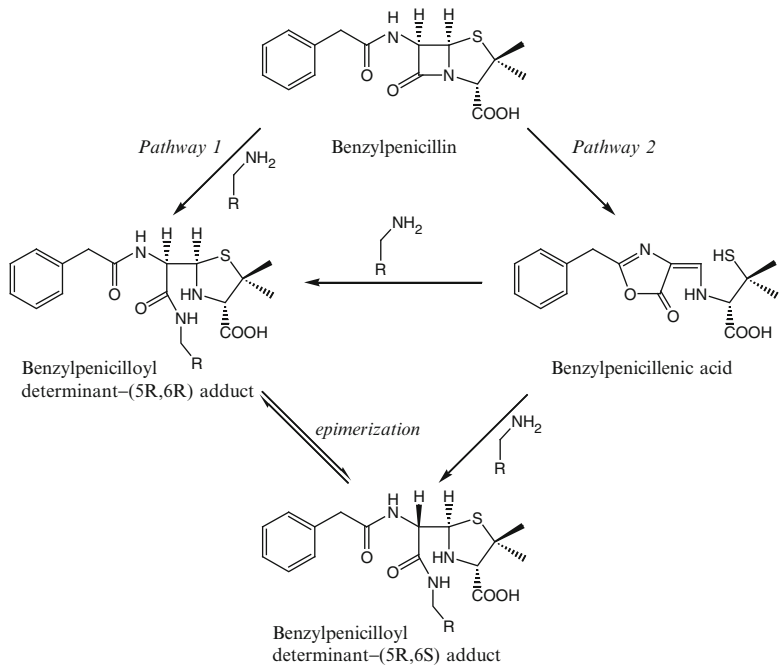


Fig. 5.5 Pathways for the formation of the penicilloyl determinant. Pathway 1: Opening of the β-lactam ring of benzylpenicillin and nucleophilic attack on protein amino groups, specifically ε-amino groups of lysine residues Lys199. Pathway 2: Benzylpenicillin rearranges to its isomer benzylpenicillenic acid which binds selectively via nucleophilic attack to Lys199 and Lys525 of human serum albumin to form benzylpenicilloyl-lysine adducts. From Xiaoli Meng, Rosalind E Jenkins, Neil G Berry, James L Maggs, John Farrell, Catherine S Lane, Andrew V Stachulski, Neil S French, Dean J Naisbitt, Munir Pirmohamed, B. Kevin Park. Direct evidence for the formation of diastereoisomeric benzylpenicilloyl haptens from benzylpenicillin and benzylpenicillenic acid in patients. J Pharmacol Exp Ther. 2011; 338: 841–9. Reprinted with permission from American Society for Pharmacology and Experimental Therapeutics

of the 5R,6R diastereomer to the 5R,6S diastereomer does not occur after the drug becomes covalently bound. This indicated that the latter diastereomer can only be formed by rearrangement of benzylpenicillin to benzylpenicillenic acid followed by covalent reaction of the acid with the lysine residues of the protein (pathway 2, Fig. 5.5) rather than via pathway 1 followed by epimerization to form the 5R,6S diastereomer. Mass spectrometric methods were also employed to detect and characterize antigens derived from the reaction of piperacillin with human serum albumin in phosphate buffer at pH 7.4. Two different haptens were detected: one hypothesized to result from hydrolysis of the 2,3-dioxypiperazine ring on the piperacillin side chain (Fig. 5.4) and the other by addition to the β-lactam ring. At low drug concentrations, modification of Lys541 occurred while at higher concentrations, up to 13 of 59 lysine residues were modified. Modified lysines at positions 541, 432, 195, and 190 were detected in the plasma of piperacillin-exposed patients with cystic fibrosis.

Structures of the degradation products of the penicilloyl determinant, viz., penamaldyl and penaldyl determinants and penicillamine, are shown in Fig. 5.6.

5.1.2.2 The Penicillenate, Penicilloic Acid, Penicillamine, and Penamaldate Determinants

Benzylpenicillenic acid, which forms readily from benzylpenicillin in aqueous solution, is unstable and is thought to be allergenic, particularly in contact skin allergy, after direct reaction with disulfides and cysteine sulfhydryl groups (Fig. 5.6). Immunization of laboratory animals with penicillenate–protein and penicilloyl–protein conjugates

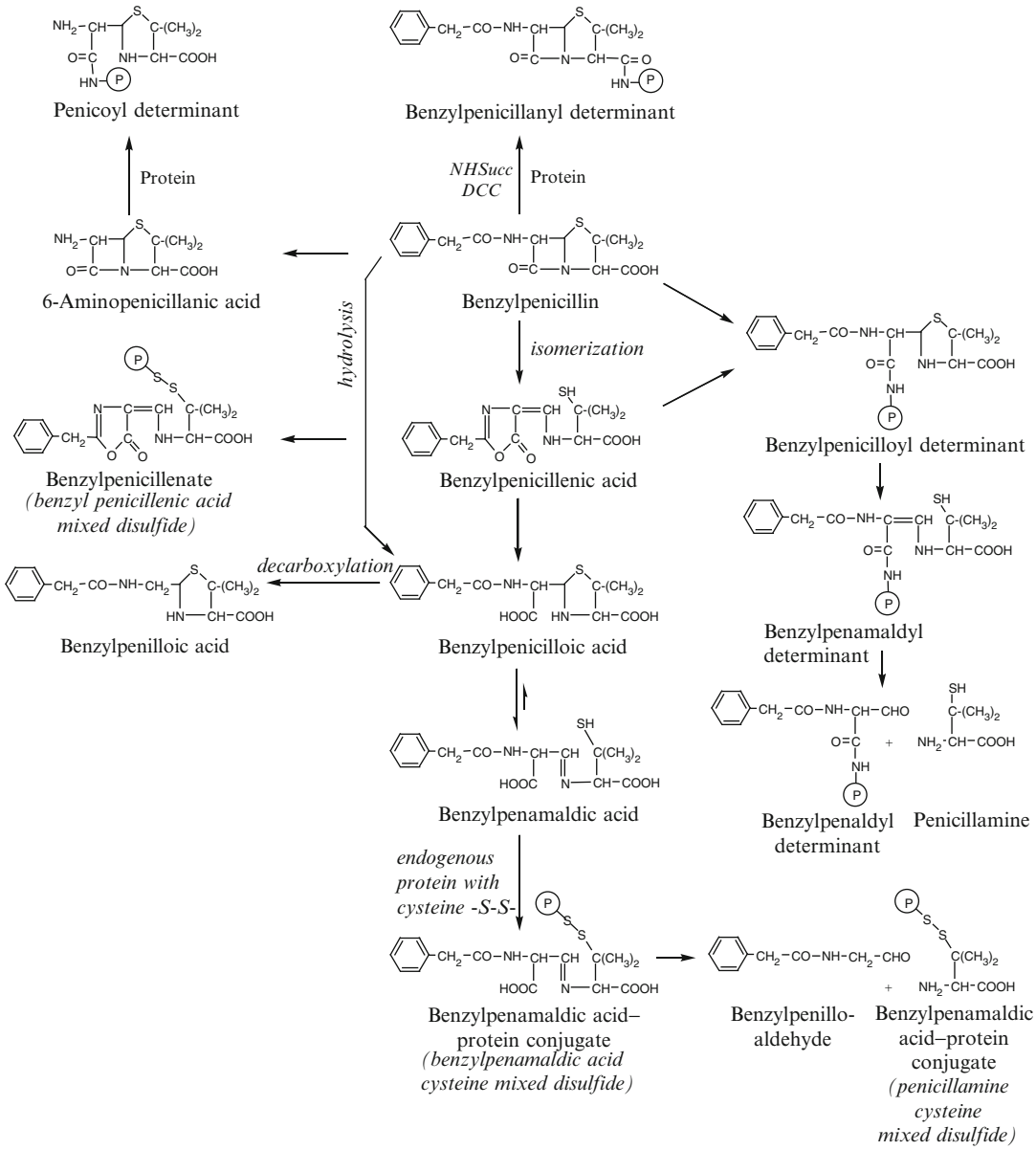


Fig. 5.6 Pathways for the formation of penicilloyl, penicillenate, penicilloic acid, penamaldate, penicillamine, and other determinants of benzylpenicillin

showed that the penicillenate and penicilloyl haptens were recognized as distinct determinants. Added evidence of the allergenicity of the penicillenate determinant was the detection of complementary IgE antibodies, although this determinant does not seem to be a clinically important allergen.

Benzylpenicilloic acid, the main hydrolysis product of benzylpenicillin, elicits wheal and flare skin reactions in some patients and was considered to be one of the so-called minor (in a quantitative sense) determinants by Levine. Decarboxylation of penicilloic acid gives rise to penilloic acid, another of the minor determinants

(see Sect. 5.1.2.4.2). It has been suggested that in vivo, penicilloic acid reacts with cystine disulfide linkages via its penamaldic acid intermediate to form benzylpenamaldic acid cysteine mixed disulfide and, then, via a penamaldate rearrangement, to penicillamine cysteine mixed disulfide and benzylpenilloaldehyde (Fig. 5.6). It is possible that these degradation products of penicilloic acid can be formed in vivo. They are chemically equipped to react with protein carriers and, theoretically at least, can function as antigens and allergens. Some evidence from skin tests studies with benzylpenicilloic acid suggests that positive skin reactions may be a response to penicillamine and/or penamaldate specificities, and skin tests with D-penicillamine-HSA and polylysine conjugates revealed a fairly high proportion of positive responses (13–41 %) in penicillin-sensitive patients. However, evidence that this group of penicillin determinants is allergenically significant remains insubstantial.

5.1.2.3 6-Aminopenicillanic Acid and the Penicoyl Determinant

6-Aminopenicillanic acid, sometimes used as a starting material for the synthesis of semisynthetic penicillins, is weakly immunogenic in laboratory animals, acts as a hapten inhibitor for reaction with the penicilloyl specificity, and does not appear to be an important penicillin allergenic structure. Any such importance 6-aminopenicillanic acid has is probably due to the penicoyl derivative formed when it reacts with protein amino groups (Fig. 5.6). Early reports of the allergenic activity of 6-aminopenicillanic acid were probably due to contamination by the penicilloyl specificity so its clinical significance and the allergenicity of the more immunogenic and antigenic penicoyl determinant need to be fully evaluated.

5.1.2.4 The "Minor" Determinants of Penicillin

Metabolites other than the penicilloyl moiety are believed to constitute about 5 % or less of administered penicillin and, together with penicillin G, are often referred to as minor determinants.

5.1.2.4.1 History

The importance of these so-called minor determinants was first demonstrated by Levine and coworkers who observed their marked association with what was described at the time as "skin-sensitizing" antibodies in penicillin-allergic patients. Levine concluded that "immediate allergic reactions to penicillin are most often mediated by skin-sensitizing antibodies of minor determinant specificities," while penicilloyl-specific skin-sensitizing antibodies were "invariably associated with accelerated and late urticarial reactions and probably mediate these reactions." The penicilloyl-specific antibodies were thought to be mainly IgG and IgM and it was suggested that these acted as blocking antibodies preventing penicilloyl-mediated immediate reactions. Two skin test solutions were originally recommended for diagnosis of penicillin allergy—benzylpenicilloyl–polylysine conjugate at a concentration of 10^{-6} M and a minor determinant mixture consisting of potassium benzylpenicillin, sodium benzylpenicilloate, and sodium benzylpenilloate all at a concentration of 10^{-2} M. The penicilloyl–polylysine conjugate contained 20 lysine residues with 13 of them coupled to the penicilloyl hapten and the remaining lysines succinylated. In comparative skin tests on penicillin-allergic patients, it became clear that patient responses were heterogeneous—some reacted only to benzylpenicillin, only to penicilloate, or only to benzylpenicilloyl-polylysine or any combination of two or more of the test reagents. In one of the original clinical studies on the minor determinants, 26 patients selected for a positive skin test reaction to one or more of potassium benzylpenicillin, sodium benzylpenicilloate, and sodium benzylpenilloate (all at 10^{-2} M) were skin tested with the major determinant benzylpenicilloyl-polylysine and with benzylpenicillin, benzylpenicilloate, benzylpenilloate and benzylpenicilloyl-amine to compare the allergenic activity of a range of minor determinants. The major determinant was positive in 46 % of patients and benzylpenicillin in 62 % while the penicilloate and penilloate preparations were positive in 85 and 73 % of patients, respectively, with the latter determinant not detecting any positive reactions missed by penicilloate. Although this seemed to indicate

that the presence of the penilloate specificity was redundant and could be left out of the mixture, it produced more intense skin reactions than the penicilloate determinant in a few patients. Penicilloyl-amine, prepared by reacting potassium benzylpenicillin with ammonia and used at 10^{-2} M, was positive in 77 % of patients, but other minor determinants, D-penicillamine, oxazolone (2-benzyl-4-sodium hydroxymethylene-(5)-oxazolone), and benzylpenilloaldehyde, were either negative in all patients or reacted with only about 10 % or less. It was concluded that to avoid missing penicillin-allergic individuals, penicillin, penicilloate, penilloate, penicilloyl-amine, and benzylpenicillin- polylysine must be used for skin testing.

5.1.2.4.2 Selection and Stability of Penicillin Minor Determinants

Although there seems to be general agreement that minor determinants should be included in skin testing for penicillin-allergic sensitivity, uncertainty remains over which of the individual compounds should constitute the "ideal" minor determinant mix. Results have shown that about 7–14 % of penicillin skin test-positive patients react only to the penicilloate specificity and not to other penicillin determinants, but some investigators believe that conclusive demonstrations of the importance of minor determinants are lacking and testing should be undertaken only with penicilloyl-polylysine and penicillin G. Most importantly, however, the chief problem preventing widespread routine evaluation and everyday diagnostic application of minor determinant mix preparations has been the highly labile nature of the reagents, particularly the penicilloate and penilloate components. For benzylpenicilloic acid, epimerization at C-5 for (5R,6S)-, (5S,6R)-, and (5R,6R)-benzyl-D-penicilloic acids is well known and likewise (5R,6R)-benzyl-d-penicilloate and (5R)-benzyl-D-penilloate were found to be labile in aqueous solution, giving rise to a mixture of diastereoisomers. In fact, aqueous minor determinant mix solutions are too labile at room temperature to use other than immediately after preparation. Solutions stored frozen are stable for at least 9 days while those at 4 °C can probably be reused for a limited time since ~85 % of the original activity is retained 3½ h after preparation. Clearly, the solution to the stability problem with the minor determinants is the preparation of freeze-dried compounds that can be stored dried in the form of single-dose ampules and opened and used only when needed. Progress in achieving this has been made (see Sect. 5.1.5.1).

The apparent general acceptance of testing with penicilloyl–polylysine conjugate and a minor determinant mix has obscured some aspects of the recognition of penicillin allergenic determinants that are still poorly understood and defined. Over 40 years ago Levine believed that "the haptenic determinant specificity of skin reactivity to penicillin is not known" and that the "specificity appears to be toward a hapten other than the BPO-[benzylpenicilloyl-] group, which is formed from penicillin but which is not formed from the penicilloate–penilloate group of compounds. Its identity has not yet been determined." He was also aware that, with more research, the need for additional minor determinants may become evident and, unlike the major determinant, none of the minor determinants had been covalently linked to a carrier to produce more effective skin test reagents by forming multivalent hapten conjugates. This remains the case today.

Attempts to identify penicillin metabolites have continued over the years by employing a range of techniques including thin layer chromatography, HPLC coupled with UV detection, NMR, and mass spectrometry (MS), but the need for large amounts of sample, lack of sensitivity in detecting trace amounts of metabolites, and poor information of fragmentation for analysis has not always led to the hoped-for progress. Application of newer MS-based approaches, however, such as the recent application of data-dependent liquid chromatography/multiple stage tandem MS, revealed seven minor metabolites of penicillin G in human serum. As well as the already known penicilloate and penilloate structures, other compounds identified were hydroxypenicilloate and glucuronide conjugates of penicilloate and three other reactive metabolites. Such methods may

help to provide a full definition of penicillin breakdown products and ultimately aid the selection of an optimal group of minor determinants for diagnostic application.

5.1.2.5 The Penicillanyl Determinant

Reaction with the C-3 carboxyl group of penicillins produces penicillanyl derivatives, but following administration of the drug, the penicillanyl determinant formed by covalent interaction with a protein carrier (Fig. 5.6) is unlikely to occur under physiological conditions. This almost certainly accounts for the lack of interest shown in the penicillanyl determinant despite a small number of promising claims made for its diagnostic potential. Early immunization studies with laboratory animals revealed that the resultant anti-penicillanyl antibodies did not cross-react with the penicilloyl determinant, but the antibodies did recognize penicilloyl protein conjugates when the acyl side chains of the penicillanyl immunogen and penicilloyl conjugate were similar. Hapten inhibition results, including some with the cephalosporin cephalothin, confirmed the importance of the side chain structure in determining specificity of the antigen, and this finding, together with results showing some antibody recognition of the β-lactam and thiazolidine rings, proved a forerunner for later immunochemical findings with sera from penicillin-allergic patients discussed below in Sect. 5.1.3. Rabbit antibodies to the penicillanyl determinant bind the parent drug strongly and the determinant in solid phase form is effective for the detection of penicillin-reactive IgE antibodies in patients' sera (see Sect. 5.1.6.1). In our laboratory, we compared penicilloyl and penicillanyl poly-L-lysine and HSA conjugates for this purpose. Benzylpenicilloyl and amoxicilloyl conjugates with poly-L-lysine and HSA were prepared by the addition of 0.5 M potassium carbonate at pH 11, passage through Sephadex G-25, and dialysis followed by characterization by the penamaldate assay and ^{1}H NMR spectroscopy. The "-anyl" determinants were prepared with *N*-hydroxysuccinamide and *N*,*N*′-dicyclohexycarbodiimide. To avoid self-condensation, the amino group of amoxicillin was protected (Boc protection). ^{1}H NMR spectra gave clear indication that the β-lactam ring was present and intact. Recognition by IgE antibodies was significantly greater for the "-anyl" determinants of benzylpenicillin and amoxicillin than for the "-oyl" determinants with ratios of 18.3 and 2.3 for benzylpenicillin and amoxicillin, respectively (Table 5.2). A similar comparison of skin tests does not appear to have been done. From results obtained in some of the earliest studies employing different penicillin determinants in skin tests on penicillin-allergic patients and reinforced in quantitative immunochemical and cellular investigations, it is clear that the immune response to penicillins is heterogeneous. The penicillanyl determinant is stable, easy to prepare and characterize, and retains the thiazolidine ring, β-lactam ring, and acyl side chain intact. It thus provides a potentially valuable antigen for studies of the

Table 5.2 Results of tests[a] for the detection of serum IgE antibodies to benzylpenicillin and amoxicillin. Comparison of results obtained with penicilloyl and penicillanyl solid phases

Test result	*N*	Percent (%)	Ratio positive A/O
Sera positive to both benzypenicillin and amoxicillin	95	7.4	–
Sera positive to benzypenicillin and negative to amoxicillin	22	1.7	–
Sera negative to benzypenicillin and positive to amoxicillin	44	3.4	–
Sera positive to BPO and negative to BPA	3	0.2	–
Sera negative to BPO and positive to BPA	55	4.3	55/3 = 18.3
Sera positive to AmoxO and negative to AmoxA	29	2.2	–
Sera negative to AmoxO and positive to AmoxA	68	5.3	68/29 = 2.3

From Baldo BA. Diagnosis of allergy to penicillins and cephalosporins. Structural and immunochemical considerations. Allergy Clin Immunol Int. 2000;12:206. Reprinted with permission from © 2000 Hogrefe & Huber Publishers (now Hogrefe Publishing). http://www.hogrefe.com

BPO benzylpenicilloyl determinant, *BPA* benzylpenicillanyl determinant, *AmoxO* amoxicilloyl determinant, *AmoxA* amoxicillanyl determinant

[a]1,290 subjects

allergenic recognition of penicillins at both the humoral and cellular levels.

5.1.2.6 Recognition of Penicillin Acyl Side Chains as Allergenic Determinants

The side chains (R substituents) of the most important penicillins are shown in Fig. 5.4. Early immunization studies with laboratory animals showed that the acyl side chain of penicillins elicits the production of complementary antibodies and there is an extensive literature on the production of such antibodies and subsequent specificity investigations undertaken. For our purposes, we are interested in the allergenic contribution, if any, of side chain structures. As long ago as the early 1960s it was appreciated that the side chain of penicillins plays a large part in the specificity of immunological reactions to the drugs. Side chain antigenicity is easily seen at the clinical level in the marked increase in recent years of patients allergic to amoxicillin and/or ampicillin but tolerant of the parent drug. In the early 1980s it was shown that the addition of some semisynthetic penicillins such as ampicillin, ticarcillin, methicillin, and piperacillin to the battery of skin testing reagents increased the rate of positive skin tests and, importantly, detected positive reactions to the semisynthetics in some patients who were skin test negative to benzylpenicillin. With the marked increase in administrations of ampicillin and amoxicillin, immunologic and provocational evaluations revealed increasing numbers of patients responsive only to the semisynthetic penicillins. Although not necessarily reflective of clinical relevance, clear IgE immunologic recognition of some different penicillin side chain substituents was clearly demonstrated in quantitative immunochemical direct binding and inhibition immunoassays with penicillin-solid phase complexes. For example, sera from some patients showed preferential recognition of ticarcillin even though other regions of the penicillin structure also bound IgE antibodies (Fig. 5.7). These results could only be explained by recognition of the ticarcillin R substituent by a population of penicillin-reactive IgE antibodies. Further clear-cut evidence that side chain groups are the dominant allergenic determinant in some immediate allergic reactions to penicillins was obtained from investigations on a number of patients who reacted to penicillins with a phenylisoxazolyl R substituent. For example, in two patients who experienced flucloxacillin-induced anaphylaxis confirmed by obvious clinical features of the reactions, history, skin testing (Fig. 5.8), and detection of drug-reactive IgE antibodies, quantitative hapten inhibitions revealed potent IgE antibody reactivity with flucloxacillin as well as pronounced reactivity with three structurally

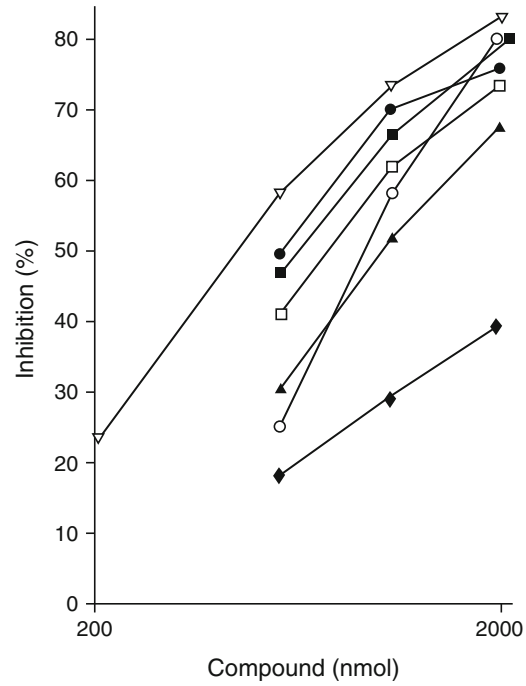


Fig. 5.7 Example of preferential recognition of a penicillin side chain (R) structure, in this case the 3-thiophene moiety of ticarcillin, and of cross-reactivity with other penicillins, by serum IgE antibodies in the serum of a penicillin-allergic patient. Quantitative hapten inhibition by β-lactam drugs of IgE binding to a ticarcillin-Sepharose solid phase: (*open inverted triangle*) Ticarcillin; (*filled circle*) ampicillin; (*filled square*) phenethicillin; (*open square*) amoxicillin; (*open circle*) benzylpenicillin; (*filled triangle*) piperacillin; (*filled diamond*) cephalothin. From Harle DG, Baldo BA. Identification of penicillin allergenic determinants that bind IgE antibodies in the sera of subjects with penicillin allergy. Mol Immunol 1990; 27: 1063. Reprinted with kind permission from Elsevier Limited

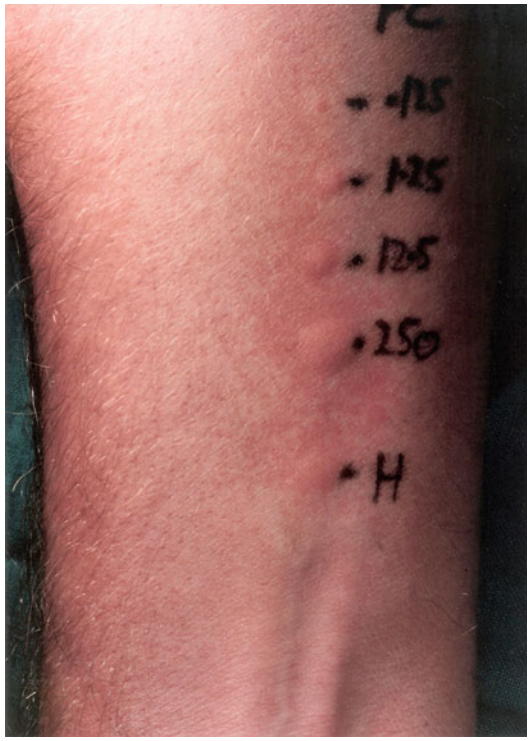


Fig. 5.8 Skin test results showing wheals following prick testing with flucloxacillin of a patient who experienced anaphylaxis after ingestion of one 500 mg capsule of the penicillin. Positive responses to the drug were obtained in the range 0.125–250 mg/ml and to histamine (H), 10 mg/ml. No wheals resulted following prick testing with Pre-Pen (penicilloyl-polylysine, Kremers-Urban, 6×10^{-5} M penicilloyl) and benzylpenicillin at 0.3, 3, 30, and 600 mg/ml. From Baldo BA et al. Detection and side chain specificity of IgE antibodies to flucloxacillin in allergic subjects. J Mol Recogn 1995; 8: 171. Reprinted with permission from John Wiley and Sons

related penicillins containing a phenylisoxazolyl side chain, viz., oxacillin, cloxacillin, and dicloxacillin (Fig. 5.9). Analysis of the inhibition results showed recognition of the 3-(2-chloro-6-fluorophenyl)-5-methyl-4-isoxazolyl group of flucloxacillin by some IgE antibodies and that the 5-methyl-3-phenyl-4-isoxazolyl group, with or without halogen substituents, accounted for the reactivity of other antibodies and for the strong cross-reactions seen with dicloxacillin, cloxacillin, and oxacillin (Figs. 5.9 and 5.10). On a molar basis, and depending on the individual patient, the di-halogenated compounds, flucloxacillin and dicloxacillin, were from about 800 to more than 13,200 times as potent an inhibitor as benzylpenicillin, clearly showing overwhelming recognition of the side chain with little or no recognition of the rest of the penicillin molecule.

Allergic recognition by some patients of side chain determinants highlights the importance of including different individual penicillins in the battery of penicillin skin test reagents.

5.1.3 Heterogeneity of IgE Antibody Responses to Penicillins and the Spectrum of Penicillin Allergenic Determinants

It was over 40 years ago that Levine pointed out the need for the identification of haptenic determinants of allergenic drugs and although he and numerous other investigators since have helped to place the penicillins near the top, if not at the head, of a list of the best defined allergenic determinants on drugs, information on the fine structural detail of allergic recognition of penicillins remains deficient. In considering the number and importance of penicillin allergenic determinants, two points recognized in the early years of research on the drug and its breakdown products are highly relevant. The first is the persisting belief that the determinant(s) responsible for the skin-sensitizing capacity of penicillins is not mainly due to the penicilloyl group and the second is the heterogeneity of the allergic response to penicillins. Early research on the allergenic properties of penicillin, its metabolites, and degradative products was hampered by lack of knowledge of both the so-called reagins mediating skin and other reactions and their hapten specificities. The research effort was consequently primarily directed toward the in vitro identification of the main penicillin metabolites and breakdown products and even when the identification of determinants was pursued via antibody recognition studies, it was generally done with heterologous antisera prepared in laboratory animals. Such antisera almost always demonstrate heterogeneity of the humoral immune response, a potential problem that can often be

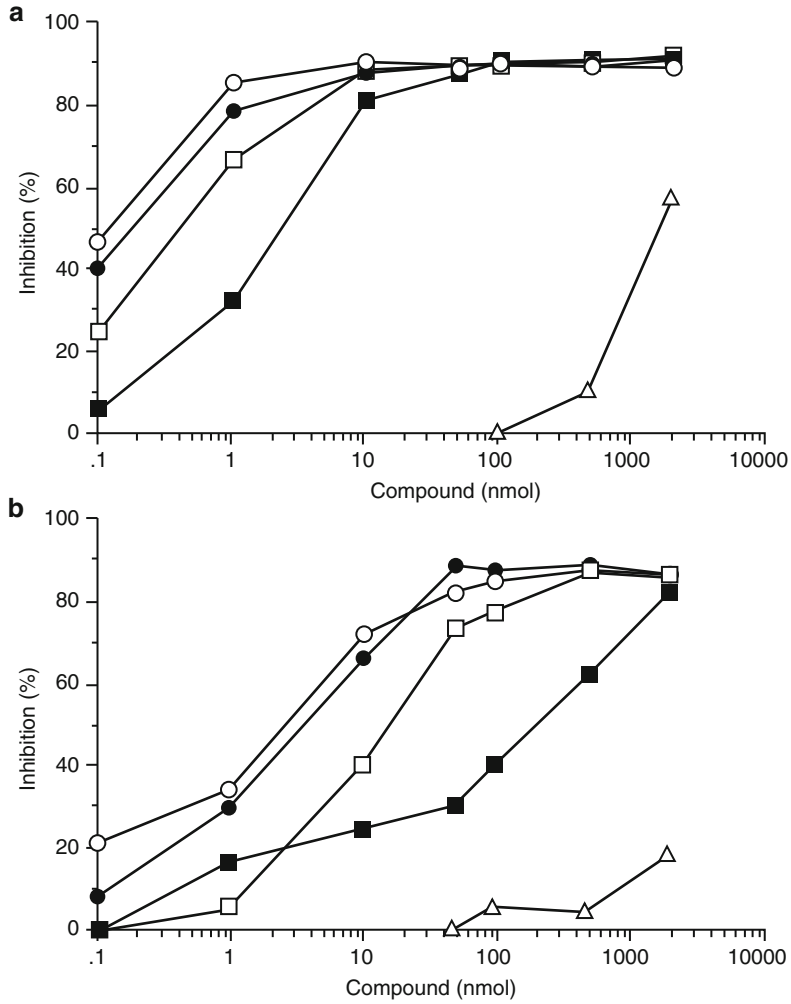


Fig. 5.9 Demonstration of preferential recognition of side chain (R) groups on isoxazolyl penicillins by IgE antibodies in the sera of two patients who experienced anaphylaxis to flucloxacillin. Quantitative hapten inhibition by some β-lactams of the binding of IgE to a flucloxacillin-solid phase covalent complex: (**a**) patient with skin test results shown in Fig. 5.8. (**b**) Results with second patient's serum. (*open circle*) Dicloxacillin; (*filled circle*) flucloxacillin; (*open square*) cloxacillin; (*filled square*) oxacillin; (*open triangle*) benzylpenicillin. From Baldo BA et al. Detection and side chain specificity of IgE antibodies to flucloxacillin in allergic subjects. J Mol Recogn 1995; 8: 171. Reprinted with permission from John Wiley and Sons

overcome by the production of a spectrum of monoclonal antibodies. Application of this strategy to benzylpenicilloyl–protein conjugate delineated three major determinants—the side chain structure, a compound determinant made up of the amide group on the penicillin molecule connected to amino acid residues of the carrier protein, and the thiazolidine ring.

In relation to immediate allergic reactions, few studies employing human sera with IgE antibodies to penicillin determinants have been undertaken with the aim of identifying the most important allergenic structural features, and for delayed reactions, such studies have been even rarer (as is the case for most drug allergens). It can be argued that an approach directed at identifying the structures recognized by the antibodies mediating the immediate allergic reactions is a more direct and clinically relevant one than the potentially more hit-or-miss strategy of first identifying a break-

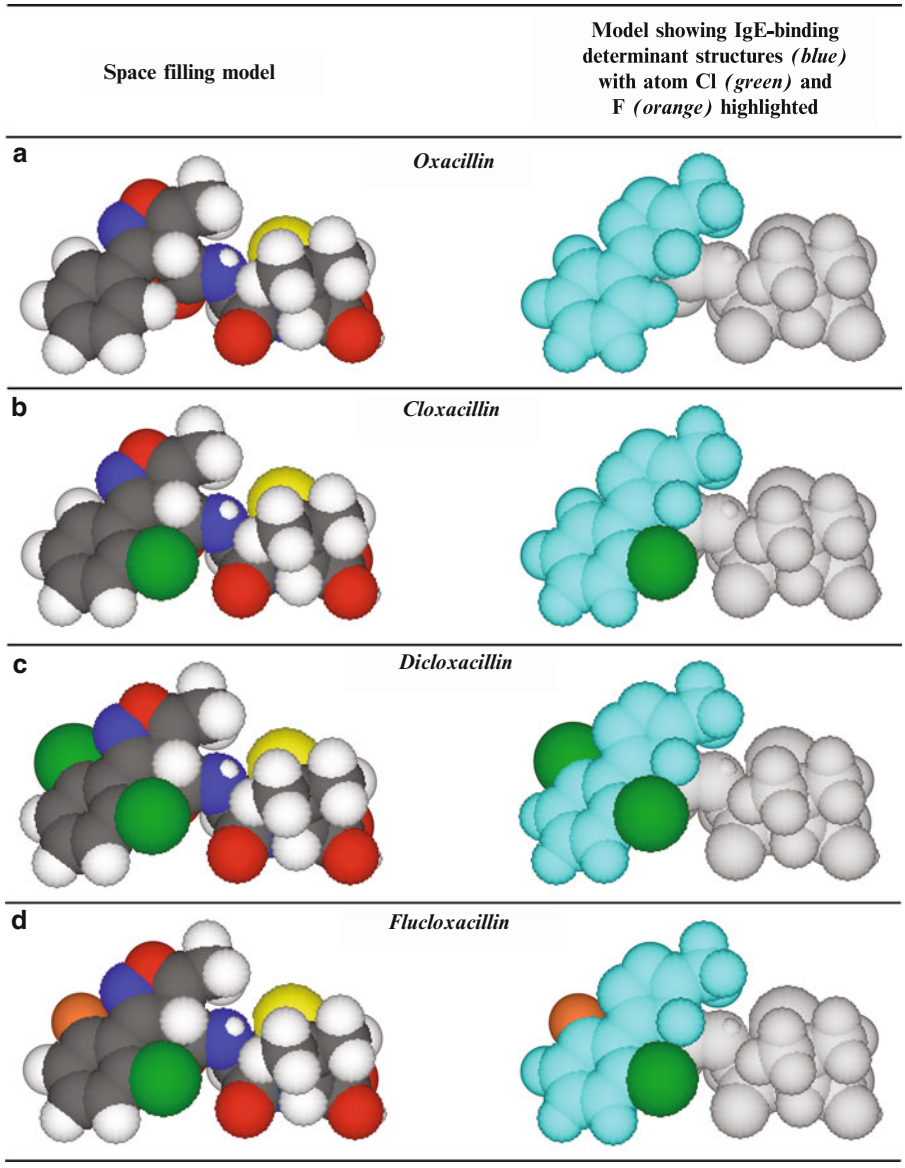


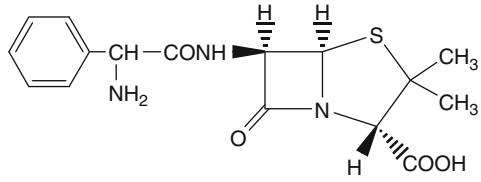
Fig. 5.10 Space-filling CPK three-dimensional molecular models showing the structures and IgE antibody-binding regions (colored *blue*, *green*, and *orange*) on the isoxazolyl penicillins oxacillin, cloxacillin, dicloxacillin, and flucloxacillin (see Fig. 5.9). Chlorine atom is *green*, *fluorine*, *orange*

down product and then accumulating enough of it to use in tests on allergic patients. An additional risk with the latter approach arises if an allergenically important metabolite present in only trace amount remains unidentified. By identifying drug allergenic structures complementary to combining sites of IgE antibodies, only the structures relevant to the stimulated allergic responses in patients are involved and it is possible to build up a full picture of the spectrum of allergenically important structural features *recognized in patient responses* to the drug. The same general strategy of identifying the determinants via the complementary immune receptors on cells can be employed in cell-mediated responses to drugs. Examples of this approach in the investigation of

T cell-mediated delayed-type hypersensitivity with associated HLA alleles, e.g., with abacavir and carbamazepine, are beginning to accumulate (see Sect. 3.4).

When the specificities of anti-penicillin IgE antibodies from patients allergic to β-lactam drugs were first studied, one of the most obvious recorded findings was the marked heterogeneity of the immune response, a feature often pointed out by early investigators but seemingly little appreciated in recent years when we have seen a heavy emphasis on clinical aspects and skin testing with the available reagents. IgE antibodies in the sera of patients allergic to β-lactam antibiotics detect a spectrum of antigenic specificities and IgE in the sera of different allergic patients show heterogeneous recognition and cross-reactive responses. It has been known for many years that some allergic patients have more than one population of β-lactam-reactive antibodies in their serum. In 1968, evidence was presented for up to eight different populations of skin-sensitizing anti-penicillin antibodies with different binding specificities. Quantitative hapten inhibition investigations employing sera from penicillin-allergic patients in radioimmunoassay experiments with semisynthetic penicillins, the parent molecule, and a range of carefully selected structural analogs often reveal antibody cross-reactivity and recognition of more than one structural domain on penicillin molecules. Results obtained with the semisynthetic ampicillin illustrate the point. Some antibodies recognized discrete regions of the ampicillin molecule such as the side chain only or the thiazolidine ring only while others were shown to have combining sites complementary to compound structures made up of the side chain with the β-lactam ring, the combination of the β-lactam and thiazolidine rings, or the whole molecule (Fig. 5.11). As well as identifying a spectrum of complementary antibody combining sites recognizing "broad" combinations of groups of atoms such as ring structures or even the entire molecule, the methodology sometimes detects antibodies with the capacity to distinguish fine structural features on different β-lactam drugs. Good examples of this are the demonstration of IgE to benzylpenicillin that cross-reacted with the cephalosporin cephalothin (see Sect. 5.2.4.2.2) and the detection of serum IgE antibodies in the sera of allergic patients that distinguished amoxicilloyl and amoxicillanyl determinants. In the latter study, antibodies from a patient who experienced anaphylaxis following an oral dose of amoxicillin reacted only with the amoxicilloyl determinant while IgE from a patient with possible penicillin allergy involving urticaria and angioedema showed multiple reactivities with penicilloyl and penicillanyl determinants of different penicillins but not with the amoxicilloyl determinant. The explanation for the recognition differences shown by the two sera lies in the different possible configurations of the amoxicilloyl- and amoxicillanyl-polylysine conjugates employed as drug-solid phases. Reaction of antibodies with the amoxicilloyl but not the amoxicillanyl conjugate reflected antibody recognition of both ends of the amoxicilloyl molecule, that is, with the aminobenzyl portion of the side chain (and perhaps with little or no recognition of the attached ring hydroxyl) and the thiazolide ring. These antibodies could not be detected with the amoxicillanyl conjugate formed by coupling through the thiazoline ring carboxyl group (Fig. 5.12). Reaction of the antibody from the second patient with the amoxicillanyl but not the amoxicilloyl conjugate reflected clear and strong antibody specificity for the aminohydroxybenzyl side chain, and especially for the 4-hydroxy substituent, which is accessible for binding in the "-anyl" but not the "-oyl" conjugate form. With the amoxicilloyl conjugate where linkage of the drug is through the open β-lactam ring, rotation and flexibility around C-6 and C-7 allow the possibility of close steric association between the side chain and the peptide carrier (Fig. 5.12). Such close association creates the possibility for

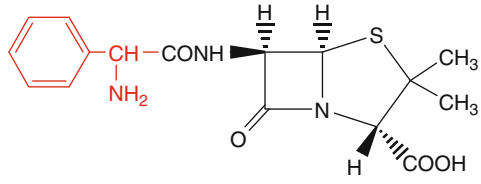
Fig. 5.11 (continued) allergic sera recognize the whole ampicillin molecule rather than parts of the structure. From Baldo BA. Diagnosis of allergy to penicillins and cephalosporins. Allergy Clin Immunol Int 2000; 12: 206. Reprinted with permission from © 2000 Hogrefe & Huber Publishers (now Hogrefe Publishing. http://www.hogrefe.com)



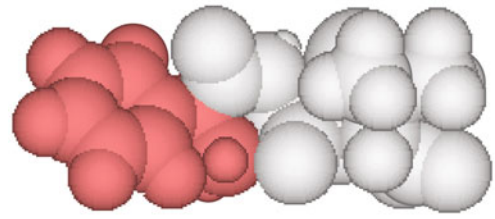
Ampicillin

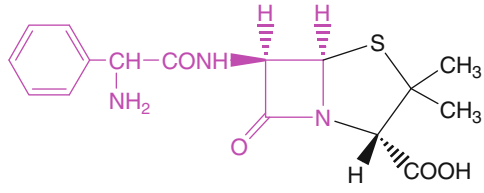
ALLERGENIC DETERMINANTS HIGHLIGHTED

MODEL WITH DETERMINANT STRUCTURES HIGHLIGHTED

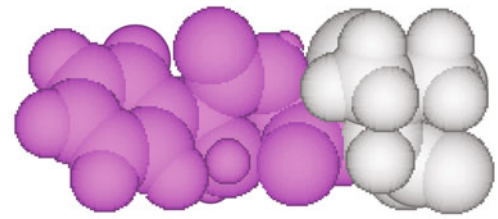


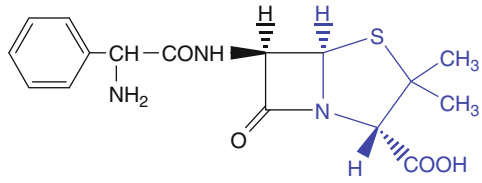
Aminobenzyl side chain



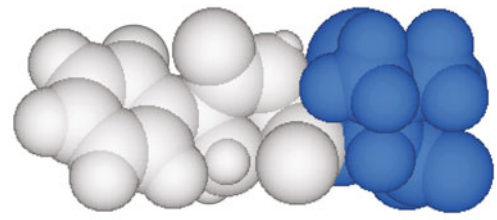


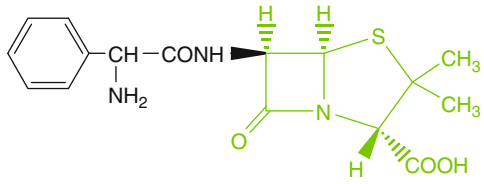
Side chain and β-lactam ring



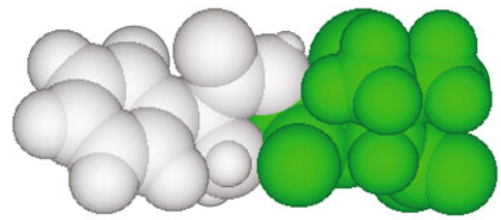


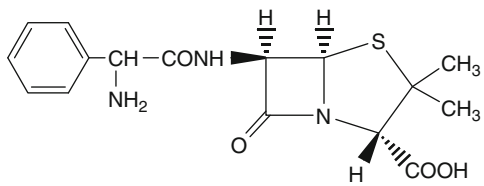
Thiazolidine ring





β-lactam and thiazolidine rings





Whole molecule

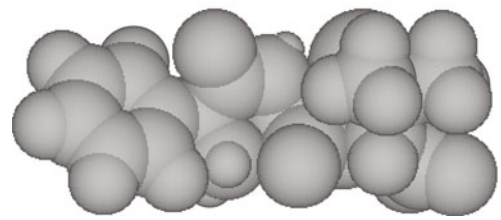


Fig. 5.11 Two-dimensional structures and three-dimensional CPK models showing the spectrum of allergenic determinants on ampicillin. Regions on the ampicillin molecule complementary to combining sites of ampicillin-reactive IgE antibodies in the sera of patients allergic to the aminopenicillin are *highlighted*. Antibodies in some

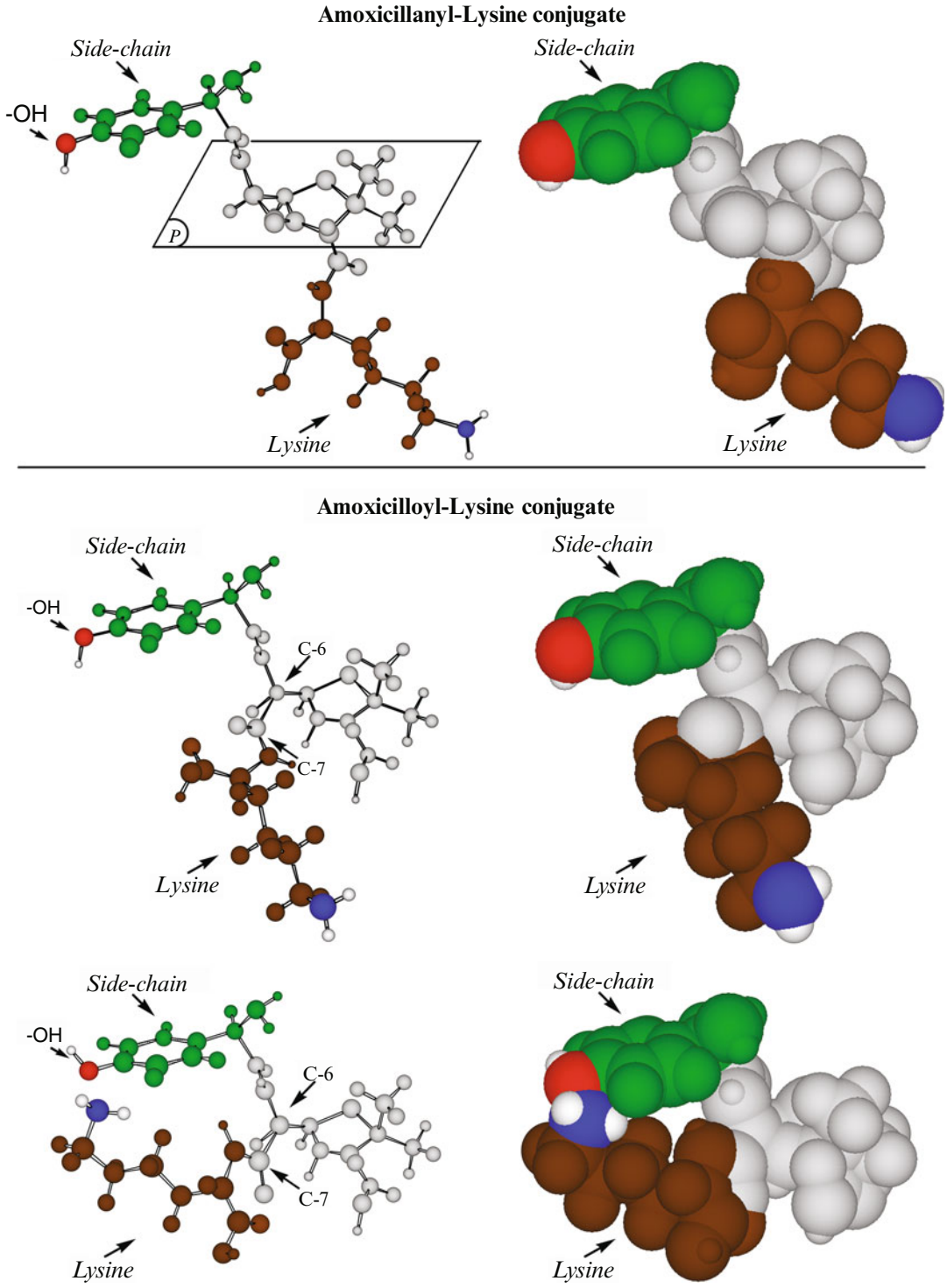


Fig. 5.12 Models (ball-and-stick, *left-hand side*, CPK space-filling, *right-hand side*) showing some possible configurations of amoxicillanyl- and amoxicilloyl-lysine conjugates. In the amoxicillanyl form, the still intact β-lactam ring confers rigidity on the molecule with the linked peptide at C-2 (shown here attached to a single lysine) below the plane (P), and the C–NH bond at C-6 above the plane, making close association of the

H-bonding involving the side chain hydroxyl group and this results in hindered access of antibody to this structure. With this patient, diagnostic employment of the penicilloyl specificity only would have produced an erroneous picture of the patient's true fine structural recognition sensitivity.

Of course, the question of the *clinical relevance* of antibody responses to drugs detected in vitro is pertinent to any discussion of the application and results of drug-specific IgE tests for the diagnosis of drug allergies. The detection of penicillin-reactive IgE antibodies may prove recognition and even sensitization to a β-lactam structure(s) but not necessarily the existence in the patient of allergy as a clinical disease. However, while the presence of a population of drug-reactive IgE antibodies does not guarantee type I allergic sensitivity, such sensitivity does not seem to occur in the absence of IgE antibodies (but see Sects. 3.2.7 and 5.2.4.3.2).

5.1.4 Risk Factors for Immediate (Type I) Reactions to Penicillins

5.1.4.1 General Risk Factors

Young and middle-aged adults appear to be at greatest risk of acute allergic reactions to penicillins, although the elderly may not cope as well with a reaction due to a generally poorer state of health and children may generally have a lower cumulative exposure to the antibiotic. It also appears that IgE-based sensitivity may wane quicker in children with one study showing a 33 % reduction in skin test positivity to penicillin 1 year after initial testing. While some studies have demonstrated a higher frequency of positive skin tests in atopic individuals, others have detected no difference. However, a higher frequency of atopy has been shown in patients who had a fatal reaction to penicillin so atopic individuals who are also allergic to penicillins may be at increased risk of anaphylaxis to the drug. Data clearly shows that more allergic reactions to penicillins occur following parenteral than after oral administration and this is in keeping with the well-known facts that anaphylaxis is a key risk of peripheral IV therapy and that the parenteral route of administration increases the severity and frequency of an anaphylactic reaction. The risk is also higher for patients with histories of anaphylaxis and urticaria compared to those with vague, mild, or unknown histories of penicillin reactions. However, allergic reactions to drugs on first exposure are known with the frequency of this occurrence varying between different groups of drugs—for example, reactions on first exposure are commonly seen with neuromuscular blocking drugs, but there are no reports of reactions after first contact with the induction agent thiopentone. Reactions provoked by the first dose of a penicillin occur, but the question of prior exposure and its possible contribution to sensitization is a difficult one to resolve given that penicillins have been found in milk, meats, other foodstuffs, human breast milk, and other environmental sources. Patients with a history of prior reactions to penicillins have a four- to sixfold increased risk of a reaction to penicillin compared to those without a previous history to the drugs. In considering penicillin exposure and risk, the persistence of IgE antibodies to the drug is another potentially important factor. Penicillin-reactive antibodies in human sera have been shown to have half-lives from as little as 10 days to many years, suggesting that their disappearance is not simply a consequence of IgE catabolism (see also Sect. 5.1.5.3.5).

Fig. 5.12 (continued) hydroxyaminobenzyl side chain group and the peptide residues impossible. With the amoxicilloyl determinant, however, opening the β-lactam ring allows increased flexibility and rotation about C-6 and C-7 and the resultant possibility of close association between the side chain and the peptide carrier linked at C-7. Two possible configurations of the amoxicilloyl determinant are shown; the lower one demonstrates the close proximity between the hydroxy group on the side chain and the peptide residues. This close association permits H-bonding and, as a consequence, access of antibodies to the side chain of amoxicillin is hindered. From Zhao Z et al. β-Lactam drug allergens: fine structural recognition patterns of cephalosporin-reactive IgE antibodies. J Mol Recogn 2001; 14: 300. Reprinted with permission from John Wiley and Sons

5.1.4.2 Risk Factors Associated with Testing

As might be expected, the risk of sensitization and a systemic reaction is lower with the prick test than with intradermal testing. In general, the risk of a skin test-induced systemic reaction is rare, but it cannot be excluded especially in some highly susceptible subjects, for example, subjects with a previous history of anaphylaxis, uncontrolled asthmatics, pregnant women, and small children. The rate of systemic reactions induced by penicillin skin testing is said to be about 1 % (see also Sect. 5.1.5.3.2). This presumably refers to patients with a previous penicillin-induced reaction. Sullivan found none of 83 skin test-negative patients given a β-lactam immediately after skin testing experienced an allergic reaction. Although the risk appears to be small, skin testing and challenge testing can each induce resensitization to penicillins. This is so even though low concentrations of drugs are used in the tests. In fact, sensitization is believed to have resulted from even lower concentrations of penicillins in the environment. In one recent investigation of over 300 cases, 2.5 % of skin test-negative subjects became skin test positive after testing with benzylpenicillin, penicilloyl-polylysine, and minor determinant mix.

5.1.5 Skin Testing Today for Immediate Hypersensitivity to Penicillin

5.1.5.1 Historical Perspective

Skin testing for allergic sensitivity to penicillins has not proved to be free of problems with the practice being beset by difficulties of regulatory requirements, suitability of testing reagents, and interrupted supply. As outlined above, the favored skin testing reagents have their origins in the early research that identified penicillin metabolites and breakdown products some of which were introduced and used for diagnostic testing. From the earliest introductions, the major determinant, benzylpenicilloyl-polylysine, first developed in 1961, was employed for skin testing at a maximum concentration of 10^{-6} M and later, the minor determinants, potassium benzylpenicillin, sodium benzylpenicilloate, and sodium benzylpenilloate (and sometimes benzylpenicilloylamine) were each used at a concentration of 10^{-2} M. The maximum test concentration of the benzylpenicilloyl-polylysine reagent was later adjusted to 6×10^{-5} M in the USA and 5×10^{-5} M in Europe. The maximum concentration for the minor determinants in Europe was changed to 2×10^{-2} M; the minor determinant mix is not available in the USA where benzylpenicillin is used alone at a maximum concentration of 10,000 IU/ml. Note that these concentrations are the same for both prick and intradermal testing. Originally sold as a research reagent marked "not for human use," the major determinant was marketed in the USA in 1973 after a large-scale cooperative skin testing prospective study sponsored by the American Academy of Allergy. In Europe, penicilloyl-polylysine was first produced and distributed as a research reagent by the Institute of Clinical Immunology, University of Berne, Switzerland, before being registered in France in 1974. Penicilloyl-polylysine (Pre-Pen®) was withdrawn from the market in the USA from September 2000 to November 2001 and then again from September 2004 to September 18, 2009, when full regulatory approval was granted by the FDA. Production of this reagent and minor determinant mix ceased in Europe in 2005 but was replaced by a new commercial kit containing both the major determinant (5×10^{-5} M) and minor determinant mix (each component at a maximum concentration of 2×10^{-2} M). In September 2011, Pre-Pen® was approved by Health Canada. In July 2011, an agreement with global distribution rights was reached for marketing the major determinant together with a minor determinant mix currently under development in the USA. During the periods of lack of supply, some clinical and laboratory investigators produced their own major and minor skin test preparations. With the increasing usage of some semisynthetic penicillins, in particular amoxicillin and ampicillin, the increase in numbers of allergic responses to these drugs, and the realization of the allergenic importance of side chain structures, amoxicillin and ampicillin

are now often included in the standard battery of skin tests. The maximum test concentration for these drugs is usually 20 mg/ml for prick and intradermal testing. Note, however, that patterns of usage of amoxicillin and ampicillin, and consequently the numbers of allergic reactions, vary between different countries. Now, after many years of uncertainty and on–off accessibility for testing reagents, the immediate future of diagnostic skin testing for allergy to penicillin looks more secure than at any time in the past.

5.1.5.2 Experience So Far of Skin Testing with Penicillin Test Reagents

In the cooperative prospective skin testing study of 3,000 subjects (1,718 with symptoms of penicillin allergy) sponsored by the American Academy of Allergy in 1977, 19 % of the cases proved positive to benzylpenicilloyl-polylysine and/or benzylpenicillin. Fifty four percent were positive to the major determinant only, 22 % to benzylpenicillin, and 25 % to both reagents. It was in this study that the currently used concentrations of test antigens were established. Addition of penicilloic acid to the panel of test reagents in a study of 740 patients, 63 % of whom had a positive skin test to at least one of the reagents, revealed positive responses of 21 % to the major determinant, 42 % to the mixture of minor determinants, and 45.2 % to the major plus the minor reagents. Some subjects were positive only to benzylpenicillin or penicilloic acid with 14.6 % of cases in the latter group. Addition of ampicillin to the test panel provided no additional information since all ampicillin-positive patients also reacted to benzylpenicillin. These studies, together with many more investigations (including some with large numbers of patients, e.g., 5,063 subjects, 776 of whom had a history of penicillin allergy), revealed significant variations in responses to the major and minor determinants. Skin testing with only the major determinant is said to identify up to 97 % of allergic patients and testing without inclusion of the minor determinants misses from 3 to 10 % of patients. From data assembled by Weiss and Adkinson, 7–63 % of patients with a positive history of penicillin allergy have a positive skin test to either the major determinant or the minor determinant mix. Overall though, responses to penicilloyl-polylysine alone or together with the response to the minor determinant mix were positive in more than 50 % of the patients. For those with a negative history of penicillin allergy, the incidence of positive skin tests is 2–7 %. For skin test-positive patients given a therapeutic dose of penicillin, the risk of an acute allergic reaction ranges from 10 % in patients with a negative history to 50–70 % in patients with a positive history. Reactions occur rarely in patients with a negative skin test (1–4 % in one study) and any reactions tend to be mild and self-limiting. The possibility of a life-threatening reaction is said to be almost negligible and any β-lactam can be safely given. Severe allergic reactions to penicillins, such as anaphylaxis, do not appear to have been reported in skin test-negative patients. With increasing prescribing of semisynthetic penicillins over more recent years, many clinicians have supplemented their panel of tests with these drugs, particularly amoxicillin. For example, amoxicillin minor determinants (amoxicillin, amoxicilloic acid, and a derivative of diketopiperazine formed from aminolysis of the parent molecules and containing the hydroxyphenyl and thiazolidine rings) have been used on patients with immediate hypersensitivity to amoxicillin. There is some data indicating that skin test sensitivity to amoxicillin may not persist as long as the skin test response to benzylpenicillin determinants. In a 5-year follow-up investigation of cases, 40 % of the benzylpenicillin-sensitive group became skin test negative whereas all of the patients with side chain sensitivity to amoxicillin became negative. These reagents did not increase the number of patients with positive reactions to the drug. Even with increases in the administrations and allergic responses to the semisynthetic penicillins, some skin test studies have detected a significant number of patients positive to only benzylpenicilloyl-polylysine and/or benzylpenicillin minor determinant mix. A recent retrospective study of over 800 patients consulting for possible allergy to a β-lactam drug revealed that the employment of these two test

reagents also detected an additional 27.6–32 % of positive reactions in patients allergic to other β-lactams.

5.1.5.3 Some Important Aspects of Skin Testing for Penicillin-Allergic Sensitivity

For a detailed description of the background, rationale and methodology of skin testing with drugs, and for the interpretation and reading of results, the reader is referred to Sect. 4.2. Prick testing should be done first and should be followed by intradermal testing only if the prick test is negative.

5.1.5.3.1 Indications for Skin Testing

It seems prudent to skin test all patients with a history of allergy to a penicillin if, at the time, penicillin remains the indicated drug of choice. Skin testing should be carried out immediately before administration of the drug and repeated before any subsequent courses. Skin testing with penicillins or any other β-lactam is absolutely contraindicated in patients with a history of Stevens–Johnson or Lyell's syndrome (toxic epidermal necrolysis), exfoliative dermatitis, or other reactions where β-lactam drug administration is contraindicated.

5.1.5.3.2 Safety

Serious reactions and even death have been reported following skin testing with penicillin reagents, but if the test is done properly and potential dangers (such as those that might be apparent in the patient's history) are anticipated, skin testing is generally a safe procedure with a systemic reaction rate of about 1 % or less. Most systemic reactions that do occur are mild. About 2–7 % of patients with no history of reactions to a penicillin show a positive skin test and most penicillin-induced anaphylactic deaths occur in patients with no apparent history of a reaction to the drug. Severe reactions have occurred to higher than recommended test concentrations or after intracutaneous testing without first doing a prick test. Skin testing should be done in the presence of a physician capable of managing anaphylaxis and with ready access to the appropriate medications and equipment. Importantly, after an episode of anaphylaxis, skin tests may be negative for up to 2 weeks or even longer. This can have important consequences if the culprit drug was not identified. For patients who test negative after an anaphylactic episode, skin testing should be repeated after 3–6 weeks.

5.1.5.3.3 Sensitivity and Specificity of Skin Testing

To discriminate true allergic from nonallergic responses, the drug provocation test is normally used but, with penicillins, the risk of challenging a patient with both a positive history and skin test is generally considered to be unacceptable. This, of course, makes the determination of specificity and sensitivity of skin tests difficult. Although skin tests to penicilloyl-polylysine have been considered to be positive in up to 70 % of patients with immediate type I responses to penicillin, testing of 290 patients with a history of immediate allergic reactivity to penicillin (71 % anaphylaxis, 29 % urticaria) revealed skin test sensitivities of 22 % for the benzylpenicilloyl hapten, 21 % for minor determinant mix, 43 % for amoxicillin, and 33 % for ampicillin. Skin test positivity to at least one determinant occurred in 70 % of the patients, showing that 30 % of patients could be misdiagnosed without further diagnostic investigation. These results are not reassuring since even with the employment of four different determinants, skin test sensitivity was a long way short of ideal. A second unexpected and worrying finding was the number of patients with a negative skin test but a positive drug provocation test. This does not fit with the currently accepted belief that the possibility of reacting to a penicillin is negligible in subjects with negative skin tests to the major and minor determinants. To establish the specificity of skin testing for penicillin sensitivity, results from tests on subjects with known tolerance to the drug must be obtained. When this is done, specificity is generally good and in the range 97–99 %.

5.1.5.3.4 Reading Tests

Results are read 15–20 min after completing the skin test. A 3 mm wheal accompanied by erythema with a negative response to a saline control is generally taken as the threshold for a positive

prick test while a positive intradermal test result is often considered to be an *increase* in wheal size (accompanied by erythema) of 3 mm or more over the diameter of the bleb size formed following injection (usually about 2 mm). A positive intradermal test result is therefore usually a wheal with a diameter of around 5 mm or greater surrounded by erythema. Patients should be advised of the possibility of a late reaction. A positive late reaction to intradermal testing may manifest as erythema, papulation, infiltrate, eczema, and swelling. Any infiltrated erythema with a diameter greater than 5 mm should be considered a positive reaction.

5.1.5.3.5 Persistence of Skin Test Reactivity to Penicillins

Skin test reactivity to penicillins generally decreases with time. Testing has shown that skin tests carried out within 1–2 months of an acute allergic reaction to penicillin were positive 80–90 % of the time, but this was followed by a time-dependent decline—in one study, positive reactions to penicillins persisted for 7–12 months in 93 % of subjects. In another early study, Sullivan and coworkers found a positive response in 73 % of patients within 1 year, 57 % continued to show a positive reaction between 1 and 10 years, and there were still 22 % of positive reactors after 10 years. The chance of a positive skin test response therefore appears to decrease by about 10 % per year, meaning that about half the patients who had an immediate reaction to penicillin will be skin test negative after 5 years. Long-lasting IgE antibody formation to penicillins often occurs in patients who have had penicillin-induced serum sickness reactions.

5.1.6 In Vitro Tests for Immediate Hypersensitivity to Penicillins

As with other drugs, but perhaps more so, a variety of humoral and cellular investigations have been utilized over many years with the aim of aiding the diagnosis and elucidating underlying mechanisms of penicillin hypersensitivities. The most commonly and widely used in vitro diagnostic tests for penicillin-induced type I allergic reactions are essentially the same as the tests employed for the diagnosis of immediate hypersensitivities to other drugs. These tests are presented in detail in Chap. 4 and this information should be referred to before proceeding with this section.

5.1.6.1 Detection of Penicillin-Reactive IgE Antibodies

While it is clear that the prime choice of tests for the diagnosis of penicillin-induced immediate reactions is the skin test, co-employment of serum IgE tests for penicillin-reactive IgE antibodies is advisable since some cases of positive IgE tests have been seen in patients with a history of an immediate reaction to a penicillin but a negative skin test to the drugs.

Soon after the development in 1967 of the radioallergosorbent test, known as the RAST, for the in vitro detection of allergen-reactive IgE antibodies, Wide and Juhlin in Sweden applied the test to the sera of penicillin-allergic patients using solid phases prepared from benzylpenicilloyl and phenoxymethylpenicilloyl protein conjugates. IgE antibodies to the penicilloyl determinants were found in 9 of 11 patients and results from skin tests and RAST reactions agreed for positive and negative reactors. Subsequent early applications of penicillin RASTs revealed rare positive reactions to penicillamine, cross-reactivity between penicillin minor determinants and the major determinant, and the finding that the penicillanyl determinant yielded no more information than the penicilloyl determinant (compare Sect. 5.1.2.5). In perhaps the most informative of the early applications of the RAST to penicillin allergy, Dewdney's group prepared and examined thiol-linked penicillamine, benzylpenicillenic acid and the benzylpenicillanyl determinant. These reagents essentially confirmed the importance of the penicilloyl determinant, but, most importantly, the study also confirmed that the heterogeneity of the immune response to penicillins extends to the specificity of the serum IgE antibodies.

In more recent years, a number of laboratories have developed and applied their own in-house

immunoassays to detect serum IgE antibodies to the parent drug and some semisynthetic penicillins, principally amoxicillin and ampicillin. Perhaps the best known commercially available test reagents for detecting penicillin-reactive IgE antibodies are the Phadia ImmunoCAP® (Thermo Scientific) drug-solid phases for penicilloyl G and V, amoxicilloyl, and ampicilloyl determinants which are widely distributed. These assays measure specific IgE antibodies in the range 0.01–100 kUA/l with a cutoff value of 0.35 kUA/l for a positive result and levels higher than 0.1 kUA/l, indicating sensitization to the drug. One assessment of the performance of the benzylpenicilloyl and amoxicilloyl ImmunoCAP assays using sera from patients with positive skin tests to amoxicillin and/or what was described as "other benzylpenicillin-derived agents" revealed sensitivity of 54 % with a specificity of 95–100 %. While the sensitivity of the amoxicilloyl ImmunoCAP assay in tests on 29 sera from patients skin test positive to amoxicillin but negative to benzylpenicilloyl-polylysine and minor determinant mix was 41 % and 42 % of 26 skin test negative, provocation test-positive patients were positive in the immunoassay, showing that the provocations could have been avoided by doing the IgE test. In another IgE examination of sera from 58 patients who each experienced an immediate reaction to a β-lactam and had a positive skin test to at least one of benzylpenicillin, benzylpenicilloyl-polylysine, minor determinant mix, amoxicillin, ampicillin, and cephalosporins, the sensitivity and specificity of the same reagents were found to be only 37.9 and 86.7 %, respectively. A similar study some years earlier on patients with immediate reactions and positive skin tests detected penicillin-reactive serum IgE antibodies in 37 % of the patients. As pointed out by the Blanca group, immunoassay sensitivities, but not necessarily specificities, for penicillins developed in individual laboratories can compare favorably with the commercial assay with one comparison showing specificities and sensitivities of 83.3–100 % and 12.5–24 %, respectively, for the commercial assay and 66.7–83.3 % and 42.9–75 %, respectively, for the laboratory test. These figures are similar to a comparison undertaken in the authors' laboratory in 2001 when, using both penicilloyl and penicillanyl derivatives of benzylpenicillin and amoxicillin, sensitivities for the detection of benzylpenicillin- and amoxicillin-reactive IgE antibodies in the sera of 28 patients with diagnosed immediate hypersensitivity reactions to a β-lactam were 57.1 % and 78.6 %, respectively, while the corresponding figures for the ImmunoCAP assays were 35.7 % and 28.6 %. Once again, however, specificities of 80.7–87.3 % for the laboratory tests were less than the results of 86.3 and 98.2 % obtained with the commercial assays. Clearly, improvements in the IgE antibody in vitro assays are needed, especially in regard to sensitivities of the tests for different penicillins.

5.1.6.2 CAST-ELISA® and Flow-CAST®

Note that as a diagnostic test for β-lactam allergy, serum IgE determinations are claimed to be less sensitive than the cellular allergy stimulation test (CAST®) (Buhlmann Laboratories AG) which measures the release of cysteinyl leukotrienes from peripheral blood leukocytes following allergen challenge (see Sect. 4.5.3). In a multicenter study of 181 patients with a history of immediate hypersensitivity to a β-lactam, overall sensitivity with the CAST-ELISA® in skin test-positive patients was 41.7 % and 27.9 % in skin test-negative patients. When these results were considered together with Flow-CAST® (Sects. 4.5.3.1 and 4.5.3.2) results, diagnostic sensitivity increased to 64.3 % with a specificity for both tests combined of 73–92 %. Sensitivity of specific IgE determinations in the same population was 28.3 %, a figure which seems extraordinarily low. Individual specificities for specific IgE determinations, CAST-ELISA®, and Flow-CAST® were claimed to be 86.5 %, 78.7 %, and 88.9 %, respectively. In the multicenter study, a maximum sensitivity of 85–90 % was reached in 112 of 124 patients with a history of allergy to amoxicillin when all four tests, skin tests, serum β-lactam-reactive IgE assays, Flow-CAST®, and CAST-ELISA® were applied in that order to patients with a negative reaction to the previous test. On the downside, however, the increase in sensitivity was matched by a decrease in specificity. Even then, the eight

negative patients responded positively to a controlled challenge. Although it was claimed that application of so many tests can cut down the number of challenges and therefore reduce costs and patient discomfort, one wonders about the practicality of the routine extra investigations let alone the extra economic cost to so many patients and the national health care bill. A more logical, potentially effective, and sensible approach may be to put greater effort into researching β-lactam allergenic determinants with the aim of defining a more optimal set of determinants that will increase the sensitivities of the routine skin and serum IgE antibody diagnostic tests.

5.1.6.3 The Basophil Activation Test in the Diagnosis of Penicillin Immediate Hypersensitivity

There have been at least five studies of the performance of the basophil activation test in the diagnosis of immediate hypersensitivity to β-lactams. All of the studies demonstrated a sensitivity of about 50 % with specificity in the range of ~90–100 %, although sensitivities as high as 67 % and as low as 20 % were seen in an investigation in which the basophil activation markers CD63 and CD203c were compared (see Sect. 4.6.2) in the diagnosis of amoxicillin allergy. Amoxicillin induced upregulation of CD203c in 60 %, or 12 of 20 anaphylactic patients skin test positive to amoxicillin, but upregulation of CD63 was significantly lower at 20 % (4 out of 20 patients). Somewhat surprisingly, upregulation of CD203c and CD63 by ampicillin was more than amoxicillin, occurring in 67 % (8 of only 12) and 33 % (4 of 12), respectively, of the anaphylactic patients. Also disconcerting was the report of ten false positives, confirmed by negative provocation tests, with both markers.

5.1.7 Challenge (Provocation) Testing for Penicillin Hypersensitivity

This section should be read in conjunction with the discussion of challenge testing set out in Sect. 4.4.

Challenges should be performed only after prior skin testing and preferably a drug-specific IgE antibody test. If either of these tests returns a positive result that is in accordance with the patient's history, the risk precludes provocation testing. According to the ENDA (European Network for Drug Allergy) guidelines, in the first instance, skin and IgE testing should be undertaken with benzylpenicillin, and, if this is positive, the patient should be considered to be allergic to the β-lactam group of drugs. If testing with the parent penicillin is negative, the patient is then tested with the drug that provoked the reaction if it is known. A positive reaction to a known drug confirms selective allergy to the drug. When the drug is not known, and in the case of a negative reaction to a known drug, a diagnosis cannot be made and further testing should then proceed beginning with an aminopenicillin such as ampicillin or amoxicillin. Figure 5.2a, b shows an example of a rash on a patient's neck, arms, and hands that developed after the last oral challenge dose of amoxicillin. The patient had previously tested negative to the penicillin in the intradermal test. Challenges with drug and a placebo are performed in a single blind procedure by a physician able to manage anaphylaxis preferably in an intensive care setting in a hospital environment with all the necessary resuscitation facilities and medications available to handle a possible emergency (see Sect. 4.4). Provocation testing of patients with exfoliative dermatitis, Stevens–Johnson syndrome, or Lyell's syndrome is contraindicated. Intervals between increasing doses of the drug should be at least 30–60 min and progress to the next increase should not occur before each dose is clearly judged to be well tolerated. Any dose that causes systemic responses even if they are mild such as rhinitis, redness or pruritus should be repeated until tolerance is demonstrated. Administration of an antihistamine is usually enough to control the symptoms of these reactions. Any more severe reaction that looks like an allergic reaction such as swelling of the throat, wheezing, or a drop in blood pressure should be treated with appropriate measures including epinephrine, steroids, bronchodilators, etc.

Table 5.3 ENDA protocols for penicillin parenteral and oral provocation tests

Drug	Dose	Cumulative dose	Route of administration
Benzylpenicillin	10^3 IU/ml	10^3 IU/ml	IM
	10^4 IU/ml	1.1×10^4 IU/ml	IM
	10^5 IU/ml	1.1×10^5 IU/ml	IM
	5×10^5 IU/ml	6.1×10^5 IU/ml[a]	IM
Phenoxymethylpenicillin and amoxicillin	1 mg[b]	1 mg	Oral
	5 mg[b]	6 mg	Oral
	50 mg[c]	56 mg	Oral
	100 mg[d]	156 mg	Oral
	250 mg[e]	406 mg	Oral
	400 mg[f]	806 mg[g]	Oral

Interval between doses 30–60 min
1 IU penicillin = 0.6 μg
Cumulative dose needs to be adapted to children and patients with kidney or liver disease
ENDA European Network for Drug Allergy, *IM* intramuscular
[a]Cumulative dose should be no more than 10^6 IU/ml
[b]Normally 1–5 mg but 0.1–5 mg for patients with history of a severe reaction
[c]50–65 mg
[d]100–150 mg
[e]250–300 mg
[f]400–800 mg
[g]Cumulative dose should be no more than 1,000 mg

Such responses are interpreted as a positive allergic reaction and the challenge is discontinued. Table 5.3 sets out the recommended ENDA protocol for penicillin drug provocation testing with the parent penicillin given parenterally and penicillin V and amoxicillin administered orally. Suggested dose ranges for each step and maximum cumulative doses are shown. If the reaction to a penicillin is not an immediate type I hypersensitivity, reactions following dosages may occur with intervals of hours or days and this must be considered before proceeding with the next challenge step.

5.1.8 Penicillin Desensitization

This section should be read in conjunction with the presentations on desensitization in Sect. 3.5.

Although there are risks associated with desensitization to a drug, a patient may, for example, show drug resistance to a possible alternative antibiotic and there may also be the possibility of failure to control an infection by substituting a drug that provides poorer bioavailability, bacteriostatic or bactericidal action. In such cases, the risk of infection may outweigh the desensitization risks. Protocols using both the parenteral and oral routes have been developed for penicillin desensitization. Desensitization can be achieved safely by the former route, but oral challenges have caused fewer severe reactions and are generally considered to be safer. Again, patients with a history of exfoliative dermatitis or Stevens–Johnson or Lyell's syndromes should not be subjected to desensitization and the procedure should be carried out in an intensive care setting with an IV line set up, β-adrenergic antagonists discontinued, and blood pressure, pulse, and respiratory rate recorded after each dose. Note that Castells and coworkers have recommended that in addition to the exfoliative skin reactions mentioned above, patients with other reactions including maculopapular rashes, fixed drug eruptions, bullous erythema, drug reaction with eosinophilia and systemic symptoms (DRESS), transaminitis, acute interstitial nephritis, serum sickness, hemolytic anemia, thrombocytopenia, or neutropenia should not be subjected to rapid IV desensitization. Before beginning a

desensitization procedure, the patient's risk situation should be assessed and the history should be consistent with a mast cell-IgE-mediated response. Penicillin skin testing with the major and minor determinants has a high negative predictive value and these tests can be particularly useful for patients with uncertain histories. Patients with a negative skin test are usually not candidates for desensitization while those with positive tests are advised to avoid penicillins and cephalosporins, especially the first-generation drugs. Desensitization should be considered for the skin test-positive patients if administration of these antibiotics is judged to be necessary.

Protocols developed in the USA by Sullivan and his collaborators have been successfully used and adapted for many years. In one demonstration of the utility of the oral procedure using phenoxymethylpenicillin, 15 pregnant women, most infected with syphilis, were both desensitized and cured of their infections. Reactions during the desensitization process and subsequent therapy were confined to the skin and were not serious. The starting dose for desensitization is commonly about one ten-thousandth or less of a full therapeutic dose. Using penicillin in solid form and starting with a dose of 0.05 mg, Sullivan employed doubling doses at 15 min intervals in 14 steps up to a maximum dose of 400 mg and a cumulative dose of 800 mg before administering the full therapeutic dose of the drug 30 min after the last desensitizing dose. An example of a protocol for a rapid oral desensitization procedure is shown in Table 5.4. For desensitization via the IV route, doubling doses, starting with an initial dose of 0.01 mg, are administered at 15 min intervals in 17 steps until a maximum dose of 640 mg and a cumulative dose of 1,280 mg are reached. Again, the full therapeutic dose is administered 30 min after the final dose. For a patient to remain in the desensitized state, penicillin dosage will normally need to be maintained, often on a twice daily schedule. If penicillin is discontinued for 48 h or more, it is highly likely that desensitization will have to be repeated.

During the stepwise dosage procedure, any dose that provokes even a mild systemic reaction should be repeated until the patient tolerates the dose without adverse signs or symptoms. Reactions that are more serious such as hypotension, asthma, or laryngeal edema need appropriate treatment but, if the decision is made to continue with the desensitization procedure, the patient should first be stabilized before dosage is continued with one-tenth the amount per dose. Skin test responses to penicillins diminish with desensitization and may become negative. The literature contains many case reports and a few case series on rapid desensitization to antibiotics in cystic fibrosis patients where infections by *Pseudomonas aeruginosa* and antibiotic allergies are a common problem often requiring desensitization of the infected patients. These studies collectively provide important information on the feasibility, performance, and safety of β-lactam antibiotics in a population of high-risk patients with poor lung function. Rates of successful desensitization ranged from 58 to 100 %. It should be emphasized that most patients require ongoing courses of desensitization with time. In desensitizations carried out in the Drug Desensitization

Table 5.4 Rapid oral desensitization protocol[a] for patients with a positive skin test to penicillin(s)

Step[b]	Dose (mg[c])	Cumulative dose (mg[c])
1	0.03	0.03
2	0.06	0.09
3	0.12	0.21
4	0.24	0.45
5	0.50	0.95
6	1	1.95
7	2	3.95
8	4	7.95
9	8	15.95
10	16	31.95
11	32	63.95
12	64	127.95
13	125	252.95
14	250	502.95

Patient should be observed for 2 h after last dose

[a]For example, for benzylpenicillin or phenoxymethylpenicillin

[b]15 min interval between steps

[c]Doses obtained from freshly made solutions of (e.g.,) concentrations 1 mg/ml (doses 1–7) and 100 mg/ml (doses 8–14)

Unit, Brigham and Women's Hospital, Boston, 15 patients completed 52 desensitizations, seven of which involved reactions. Six patients had limited symptoms of immediate hypersensitivity and one patient experienced acute respiratory failure to ceftazidime. Successful desensitizations were obtained with benzylpenicillin, nafcillin, cefazolin, and ceftriaxone.

5.1.9 Delayed-Type Hypersensitivity Reactions to Penicillins

Delayed-type, non-IgE-mediated hypersensitivity, often manifesting as macular or maculopapular exanthemata (see Sect. 2.2.4.3 and Fig. 2.7), may occur during treatment with penicillins, particularly aminopenicillins. Incidences of hypersensitivity to penicillin range up to 10 %, and for maculopapular exanthemata occurring during therapy with aminopenicillins, the incidence is about 9.5 %. At least some of the penicillin-induced exanthemata are due to T cells and can be confirmed by skin testing with aminopenicillins. Other delayed reactions elicited by penicillins include acute generalized exanthematous pustulosis (AGEP), delayed urticaria/angioedema, exfoliative dermatitis, and the more severe bullous exanthems, Stevens–Johnson and Lyell's syndromes. Severe hypersensitivity responses to penicillin that are not primarily seen on the skin include vasculitis, hepatitis, interstitial nephritis, and pneumonitis while DRESS is a combination of skin eruptions, fever, and visceral involvement. More detailed descriptions of these drug-induced reactions and what is currently understood about the underlying mechanisms are contained in Sects. 2.2.4, 3.6.3 and 3.8.

5.1.9.1 Diagnostic Tests

5.1.9.1.1 Skin Tests

An extended presentation on skin testing is set out in Sect. 4.2.

Intradermal tests with delayed reading, patch tests, and occasionally prick tests provide the mainstay diagnostic procedures for the evaluation of delayed reactions to penicillins and other β-lactam drugs. Skin prick and patch tests on a large number of patients with a history of suspected cutaneous adverse drug reactions detected 89 positive patch tests (10.8 %), mainly to β-lactams, trimethoprim, and clindamycin, in 829 patients and 10 positive prick tests (1.1 %) in 935 patients. Eight of 298 patients (2.7 %) were patch test positive to phenoxymethylpenicillin. Challenge tests on 17 patients who were skin test positive and 229 who were skin test negative produced 14 and 22 positive results, respectively. Of the 22 (9.6 %) skin test-negative and challenge test-positive patients, 12 reacted with exanthema, seven with urticaria, and three with fixed drug eruptions. In a study designed to assess the incidence of delayed allergy during penicillin therapy and to evaluate the diagnostic potential of patch, intradermal (with delayed reading), and challenge tests, ampicillin, amoxicillin, and a small number of other penicillins were employed in tests on 259 patients most of whom had experienced maculopapular rashes. Positive patch and intradermal tests with penicillins revealed 98 (94 to aminopenicillins, four to piperacillin) patients (37.8 %) with delayed reactions. Of the 98 skin test-positive patients, 93 had experienced maculopapular rashes. Of 125 patients with negative skin tests who underwent challenges, only three reacted. The investigators concluded that patch and intradermal test positivity can provide an indication of delayed hypersensitivity to penicillins, and of the two tests, intradermal testing is the more sensitive.

In a prospective investigation of allergic cross-reactivity between aminopenicillins, phenoxymethylpenicillin, and two cephalosporins with different R1 side chains, 71 patients (57 with a history of macular or maculopapular exanthema, two with erythema multiforme-like exanthema, nine with acute urticaria, and three with unclassified symptoms) were evaluated with intradermal and patch tests. Sixty eight of the 71 patients (95.8 %) had at least one positive intradermal or patch test to ampicillin or amoxicillin, 48 reacted to the aminopenicillins only, four to the aminopenicillins and benzylpenicillin, and 16 to the three penicillins plus phenoxymethylpenicillin. From these results, intradermal and patch tests were

deemed to be reliable diagnostic tools with high sensitivity for delayed-type hypersensitivity to aminopenicillins but, because tests were sometimes positive with only one of the methods, combined use of the two tests was recommended. It has been shown that positive skin tests can be obtained with aminopenicillins years after exposure to the drugs and this was confirmed in this study with 20 of the 21 patients testing positive more than 1 year after their exanthematous reactions. Importantly, a positive test result was not always obtained with ampicillin and amoxicillin so testing with both drugs seems to be necessary.

The usefulness of the penicillin major and minor determinants in evaluating delayed reactions to penicillins was assessed in intradermal and patch tests on 162 patients who experienced delayed reactions to penicillins, mainly aminopenicillins. Positive intradermal and/or patch tests in 157 patients (96.9 %) to the responsible penicillin reagents indicated cell-mediated hypersensitivity while only 9 (5.5 %) and 17 (10.5 %) were positive to penicilloyl-polylysine and minor determinant mix, respectively, demonstrating the limited usefulness of the two benzylpenicillin determinant preparations.

Individual penicillins, penicillin determinants and their concentrations (all diluted in sterile physiological saline) used in skin tests: Benzylpenicillin at 100 and 10,000 IU/ml (0.06 and 6 mg/ml); ampicillin and amoxicillin, both at concentrations of 1–2 and 20–25 mg/ml; other penicillins, 1–20 mg/ml; cephalosporins 2 mg/ml; commercially available benzylpenicilloyl-polylysine solution (see Sect. 5.1.5.1) initially diluted 1:10 and undiluted if negative; minor determinant mix diluted 1:100 and repeated undiluted (2×10^{-2} M) if initial test is negative. For patch testing, penicillins can be used at a concentration of 5 % w/w in petrolatum. Drugs in solid form such as tablets, capsule contents, pessaries, etc., should be ground finely in a mortar and formulated in petrolatum or in saline if soluble, taking into account the ratio of active drug to nondrug components in the tablet/capsule etc.

Prick and intradermal tests with penicillins should be read after 20 min and delayed reactions in the intradermal test after 48 and 72 h. Positive thresholds are a more than 3 mm diameter wheal for prick tests and an increase in initial bleb wheal diameter of more than 5 mm for intradermal tests. Reactions after intradermal testing are documented by the diameter of the erythema, papulation, and infiltrates together with descriptions of swelling, erythema, eczema, etc., and photodocumentation if possible. For reading patch tests, see Sect. 4.2.4.3.

5.1.9.1.2 Challenge (Provocation) Tests

Often restricted by ethical considerations, drug challenge can be regarded as the best test for confirming a drug-induced delayed hypersensitivity response. Patients who are negative to all of the other tests for a β-lactam may undergo challenge testing with one-hundredth of the therapeutic dose of the β-lactam as the initial dose, and, if negative, 3 days to 1 week later, a one in ten dilution of the therapeutic dose should be given. If the response is again negative, a full therapeutic dose is administered after the same interval chosen before the second dose. All precautions set out in Chap. 4 and mentioned above must be observed and the test is contraindicated in patients with DRESS, AGEP, bullous exanthemas, Stevens–Johnson syndrome, and toxic epidermal necrolysis. Tests on patients with drug-induced vasculitis, anemia, and neutropenia are also contraindicated.

5.1.9.1.3 Delayed Reactions to Penicillins and the Lymphocyte Transformation Test

This test is discussed in Sect. 4.7.1.

Although claimed to be a useful test in the hands of some experienced with the technique, others have found it unreliable and difficult to standardize. Yet to be validated and still essentially a research tool, the test is claimed to have a sensitivity of 74 % with a rather low specificity of 85 %. One group found an overall sensitivity of 62 % and a specificity of 92.8 % in 51 patients with a well-documented history of β-lactam allergy (31 immediate reactors and 19 non-immediate). This was made up of sensitivities of 64.5 % and 57.9 % for the immediate and non-immediate groups, respectively, but the proliferative responses, expressed as stimulation indices,

were higher in the cells from the delayed group. The authors considered the test to be a useful in vitro diagnostic tool to identify subjects allergic to penicillins, especially the delayed reactors where, somewhat surprisingly, they found it superior to skin testing. Interestingly, T cell proliferative responses were seen 10 or more years after initial exposure to penicillin and without reexposure in the years between.

It has been suggested that the relatively poor sensitivity of the lymphocyte transformation test is due to background nonspecific proliferation of the cultured peripheral blood mononuclear cells and only those patients with the highest numbers of antigen-specific T cells are detected with the test. This suggestion appears to have been supported by results from a comparative study in which cells from 22 patients with well-documented histories of T cell-mediated allergy to amoxicillin were examined with the lymphocyte transformation test and an ELISPOT assay (see Sect. 4.7.3.2) for the detection of amoxicillin-specific T cells producing IFN-γ. The IFN-γ ELISPOT assay seems to distinguish between immediate and delayed hypersensitivities and may prove to be a sensitive test for improving the diagnosis of delayed hypersensitivity to β-lactam drugs.

5.1.9.2 Recognition of Penicillin Antigens by T Cells

One of the relatively recent applications of the extraordinary advancement in knowledge of cellular immune processes over the last 25 years is witnessed in the field of drug allergy and, more specifically, in studies aimed at understanding the role of T cells in delayed reactions to drugs. As occurred with studies on drug-induced immediate hypersensitivity, the drugs most commonly selected for the cellular investigations have been the penicillins since their frequency of use, high incidence of reactions, and the accumulated knowledge of their antigenic structures make them the logical choice. Early investigations on T cell clones showed that benzylpenicillin-specific clones were HLA class I or class II restricted and processing of the free drug was not required whereas benzylpenicilloyl–HSA conjugate must undergo processing to stimulate T cell clones specific for this determinant. Extension of these experiments to an examination of the specificities of the T cells revealed two different recognition patterns—one directed at the penicilloyl specificity plus the side chain structure and the other more broadly cross-reactive with recognition of the aminopenicillins, ampicillin and amoxicillin, as well as benzylpenicillin. Further investigations of the structural features recognized by benzylpenicillin-reactive T cell clones from different patients identified the benzyl side chain and the thiazolidine ring as antigenic determinants. Precise positioning of covalently bound benzylpenicillin via a lysine residue on designer peptides containing a DRB1*0401-binding motif was required to induce proliferation of penicillin-specific clones and to be necessary for optimal T cell recognition but, even more interestingly, a peptide sequence derived from a natural DRB1*1101-binding peptide with attached penicillin also acquired antigenic properties. Study of the specificity of penicillin-specific T cell clones was recently taken up and extended in experiments designed to characterize the antigen recognition profile of clones from cystic fibrosis patients hypersensitive to piperacillin. Piperacillin-responsive T cell clones (CD4+, CD4+D8+, CD8+ but mainly CD4+) secreted high levels of the Th2 cytokines IL-4, IL-5, and IL-13 as well as CCR4 or macrophage inflammatory protein-1β (MIP-1β), the chemokine with specificity for CCR5 receptors. Mass spectrometry and other investigations revealed that piperacillin bound to albumin lysines in T cell cultures at residues 190, 195, 199, 432, and 541 after 16 h incubation, but, after only 1 h, the drug was detected at only one site. These results fit with the time needed to stimulate a T cell proliferative response and demonstrate the need for high piperacillin binding for T cell activation. Attempts to stimulate clones with other penicillins and with cefoperazone which has a structurally related side chain to piperacillin and a dihydrothiazine instead of a thiazolidine ring were unsuccessful, demonstrating that the T cells specifically recognized the penicillin nucleus and piperacillin side chain structures. Piperacillin–albumin con-

jugate stimulated T cells via a MHC- and processing-dependent pathway but flucloxacillin, which reacts with the same lysine residues on albumin as piperacillin, did not stimulate T cells, indicating structural specificity of the T cell receptors expressed on the drug-specific clones. Flucloxacillin did, however, competitively reduce piperacillin binding and the piperacillin conjugate-specific T cell response, suggesting that co-administration of such a competitor might prevent allergic sensitization or even reduce the hypersensitivity response in patients with established sensitivity.

5.1.10 The Genetic Basis of Penicillin-Induced Liver Injury

Due particularly to studies from Australia and Sweden, flucloxacillin has a well-known association with severe and debilitating cholestatic liver disease. The incidence of this association is estimated to be from about 1 in 10,000–100,000; a Swedish study estimated the rate to be 1 case per 10,000–30,000 prescriptions. The female sex, patient age over 55 years, high daily dose, and taking the drug for more than 14 days seem to be associated with a higher risk of the penicillin-induced liver injury. A 1992 paper from Sweden reported 77 liver reactions "probably or possibly" induced by penicillinase-resistant penicillins, including cloxacillin and dicloxacillin as well as flucloxacillin, which were reported spontaneously to the Swedish Adverse Drug Reactions Advisory Committee. A genome-wide association study of 51 cases of flucloxacillin-induced liver injury and 282 controls showed a strong association in the major histocompatibility complex (MHC) region with a marker in complete disequilibrium with HLA-B*57:01. This association, confirmed in follow-up genotype studies, offers new insights into understanding the condition and may ultimately improve its diagnosis, but the mechanism underlying flucloxacillin cholestatic hepatitis remains incompletely understood. Some clones from patients allergic to flucloxacillin are CD8+ and positive for granzymes. Flucloxacillin forms protein adducts with human serum albumin and mass spectroscopy and other techniques have revealed up to nine modified albumin lysine residues with Lys190 and Lys212 being particularly involved. Both the parent drug and its 5-hydroxymethyl metabolite reacted with the same lysine residues. Whether such drug–protein or peptide complexes play a central role in mediating flucloxacillin-induced liver injury remains to be seen.

5.2 Cephalosporins

For the early cephalosporins, the names were spelt with a "ph" and this has continued in English-speaking countries although the UK, along with European countries, appears to be adopting International Proprietary Names which use the "cef" spelling. Cephalosporins are often referred to as first- or subsequent-(second, third, and so on) generation drugs, a classification essentially based on both the sequence of development and antimicrobial action. The newer generation drugs (and some newer first-generation drugs) tend to be spelt with an "f." First-generation drugs are predominately active against Gram-positive organisms while succeeding generations were endowed with increasing Gram-negative activity. It is arguable whether or not the "generation" classification has any clinical relevance or use.

Together with the penicillins, the cephalosporin β-lactam antibacterials are the most widely used antibiotics for the treatment of common infections. The origin of cephalosporin antibiotics dates back to 1948 with the demonstration of antimicrobial action in a fungal extract from what was then called *Cephalosporium acremonium*. The *Cephalosporium* genus is now known as *Acremonium*. The cephalosporins used in medicine today are semisynthetic derivatives of the natural antibiotic cephalosporin C. Soon after cephalosporins were introduced into clinical use, adverse reactions including apparent hypersensitivity reactions and obvious anaphylactic responses began to appear, and although their similar structural features to the penicillins

suggested that the two groups might cross-react strongly and display similar antigenic and allergenic properties, clinical manifestations, and diagnostic challenges, the reality has been somewhat different. Now, almost 50 years since the first therapeutic use of cephalosporin C in 1963, there are still a number of important outstanding questions. These include the nature of cephalosporin breakdown products and allergenic determinants; the relationship between penicillin- and cephalosporin-allergic sensitivities including their clinical cross-reactivity; the specificity and predictive value of cephalosporin skin testing; the relationship between cephalosporin-reactive IgE antibodies and clinical allergy; and the speed of decline in skin test and serum IgE positivity in cephalosporin-allergic patients.

5.2.1 Incidence of Cephalosporin Hypersensitivity and Clinical Aspects

From retrospective studies and information from pharmaceutical companies, the incidence of immune-mediated adverse reactions to cephalosporins was calculated to be 1–3 %, although a figure of 1 % is commonly seen in the literature. Dermatologic reactions, mainly rashes, urticaria, and pruritus, occur with a frequency of about 1–3 %. Maculopapular rashes that are not pruritic are generally thought to be non-IgE-mediated and therefore not a reason to avoid cephalosporins, but a rash with pruritus may be an indication of an allergic reaction. Anaphylaxis to cephalosporins is said to be rare with an incidence of up to 0.1 %, but this may be a significant underestimate since no reliable large surveys seem to have been undertaken. Apart from anaphylaxis, other immediate type I reactions induced by cephalosporins include urticaria, laryngeal edema, bronchospasm, and hypotension. Serum sickness-like reactions have been reported in children, fever and immunohematological reactions are uncommon, and severe skin reactions such as exfoliative dermatitis and Stevens–Johnson syndrome occur but again are uncommon. Early reports of a high incidence of a direct positive Coombs' test in patients receiving cephalothin with the possibility of complicating routine cross-match tests and immune hemolytic anemia have not been substantiated.

5.2.2 Clinical Aspects of Cross-Reactivity of Cephalosporins and Penicillins

Retrospective surveys of the published literature on work undertaken over 40 years ago and current findings at the time on the relationship between allergy to cephalosporins and a history of penicillin allergy revealed findings that are still widely quoted and often disputed. The incidence of patients with a history of penicillin allergy who had allergic reactions to cephalosporins was found to be 9.2 % (5.4–16.6 %) while patients who had a reaction but a negative history to penicillins showed an incidence of 1.7 % (1–2.5 %), that is, allergic reactions to cephalosporins appeared to be about 5.4 times more frequent in patients with a history of penicillin allergy. A survey of 15,987 patients treated with cephalothin, cephaloridine, cephalexin, cefazolin, or cefamandole published in 1978 revealed that 8.1 % of patients with a history of penicillin allergy had reactions compared with 1.9 % without such a history; this indicates a fourfold risk for patients allergic to penicillin. However, as pointed out at the time and since then, this large difference is probably not likely because penicillin-allergic individuals show an increased incidence of drug hypersensitivities, some reactions included as allergic reactions to cephalosporins were probably not immune mediated and a positive history of penicillin allergy may not always be reliable. Over the years, there have been many studies, large and small, of cephalosporin administration to patients with a history of penicillin allergy. These have delivered widely varying results with incidences of positive reactions up to 18 % and a risk up to about eight times that of patients with no penicillin allergy. Both these figures are likely to be overestimates, but it does seem reasonably certain that there is a significantly higher risk of a reaction to a cephalosporin in patients already allergic to a penicillin. A recent retrospective cohort study using the United

Kingdom General Practice Research Database looked at a total of over 3.3 million patients given penicillin and, in particular, at over half a million (~15 %) who were subsequently given a course of cephalosporin. Estimation of numbers of patients who experienced allergic-like events within 30 days revealed that the unadjusted risk ratio for those who had a prior reaction compared to those who did not have a reaction was 10. The absolute risk for anaphylaxis after a cephalosporin was 0.001 %, leading to the conclusion that a markedly increased risk existed for patients who had previously reacted to a penicillin, but cross-reactivity was not an adequate explanation for the increased risk. Recently it has been claimed that many patients with adverse reactions quite unrelated to immune hypersensitivities were categorized as allergic in the early surveys. For this reason as well as those mentioned above, and the early use of first-generation cephalosporins, the risk of cross-reactivity is probably closer to 0.5 % than 10 %. Second- and third-generation cephalosporins appear to carry less risk of provoking a reaction in penicillin-allergic subjects and, given the structural differences seen in the side chains, especially for the third- and fourth-generation cephalosporins (see below), this is perhaps not surprising. Some recent studies have concluded that the use of third- or fourth-generation cephalosporins in penicillin-allergic patients carries a negligible risk of cross-allergenicity.

A review of the use of cephalosporins in children with anaphylactic reactions to penicillins concluded that there were no published case reports of anaphylaxis to these antibiotics in the assessed group and, in any case, anaphylaxis to cephalosporins was extremely rare in children. A prospective study of over 1,000 children with suspected immediate reactions to cephalosporins and/or penicillins showed that 58 % were skin or challenge test positive to a β-lactam with 94.4 % positive to penicillins and 35 % positive to cephalosporins. Approximately one-third of penicillin-allergic children cross-reacted with a cephalosporin and those allergic to a cephalosporin showed an 84 % frequency of reactions to penicillins. Cross-reactivity between cephalosporins was lower than cross-reactivity observed between cephalosporins and penicillins and cross-reactivity for cephalosporins overall was highest with the first- and second-generation drugs.

5.2.3 Structures and Classification of Cephalosporin Drugs

Although the natural antibiotic cephalosporin C lacked sufficient potency for therapeutic use, demonstration of the structure of its nucleus, 7-aminocephalosporanic acid, showed that the cephalosporins had a structural basis analogous to the 6-aminopenicillanic acid core of the penicillins. Like the structurally related other major β-lactam antibiotic group, the penicillins, cephalosporins also contain a β-lactam ring with a linked side chain (R1) but with a six-membered dihydrothiazine ring instead of the five-membered thiazolidine ring found in the penicillins. In addition, cephalosporins have a second side chain group (R2), which is attached at position 3 of the dihydrothiazine ring. The general structure, structures of the two side chain groups, and the generation of some of the many cephalosporins developed and marketed for therapeutic use are shown in Fig. 5.13. Classification of the drugs into different generations has not always been observed and consistent in all countries, e.g., cefaclor is regarded as a first-generation drug in Japan but a second-generation cephalosporin in the USA. The classification of cephalosporins is based on an intended broader spectrum of succeeding generations of drugs, but this has sometimes come at the cost of decreased Gram-positive activity. However, the fourth-generation cephalosporins are said to be truly broad spectrum in antimicrobial activity.

5.2.4 Cephalosporin Antigens and Allergenic Determinants

Despite the chemical similarity of the cephalosporins and penicillins, differences in chemical reactivity and stability exist. This is most obvious when conditions to prepare the penicilloyl determinant are applied to the cephalosporins.

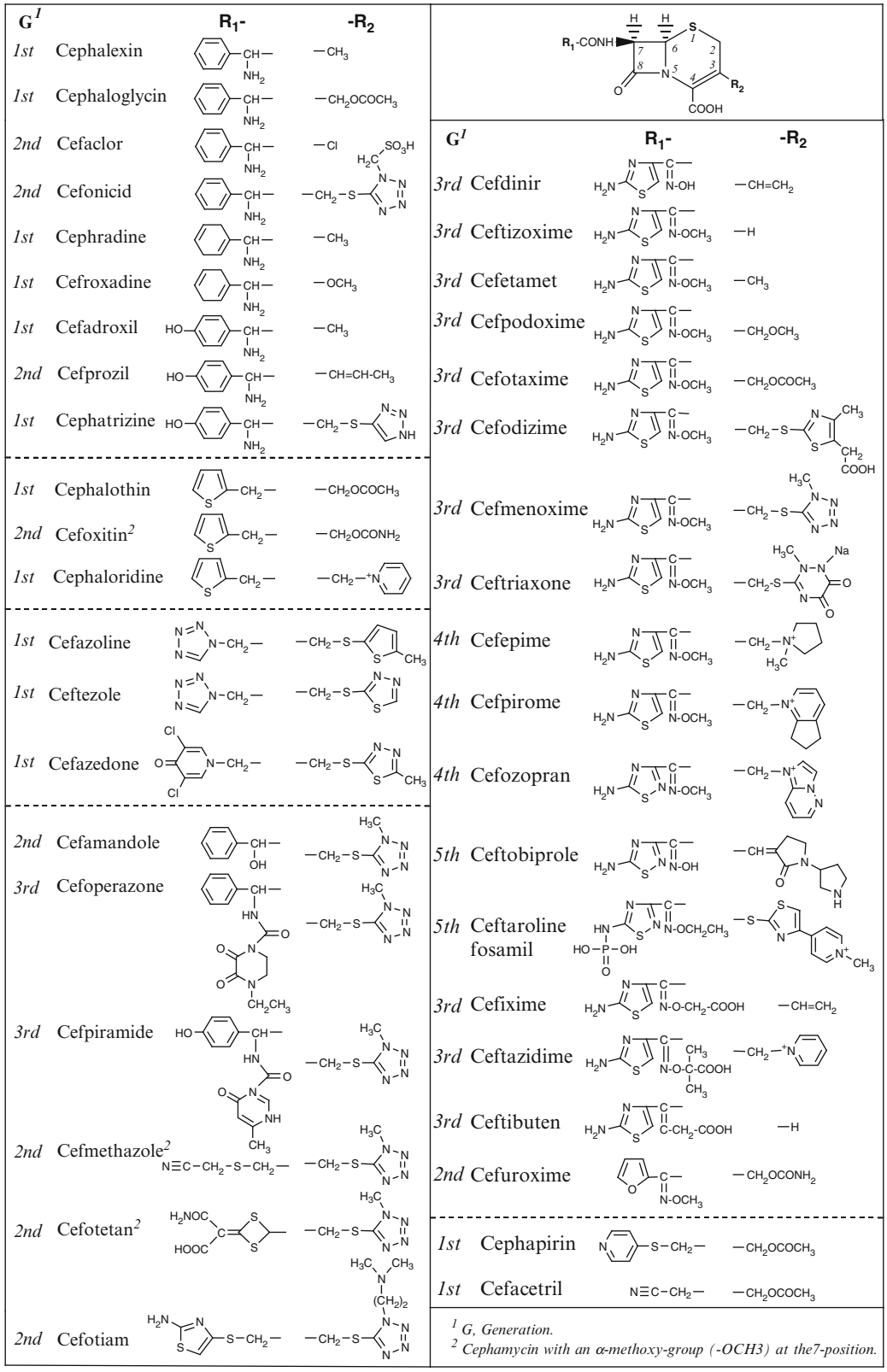


Fig. 5.13 Structures of some of the more frequently used first-, second-, third-, fourth-, and fifth-generation (G) drugs out of the large number of cephalosporins developed as potential antibacterials

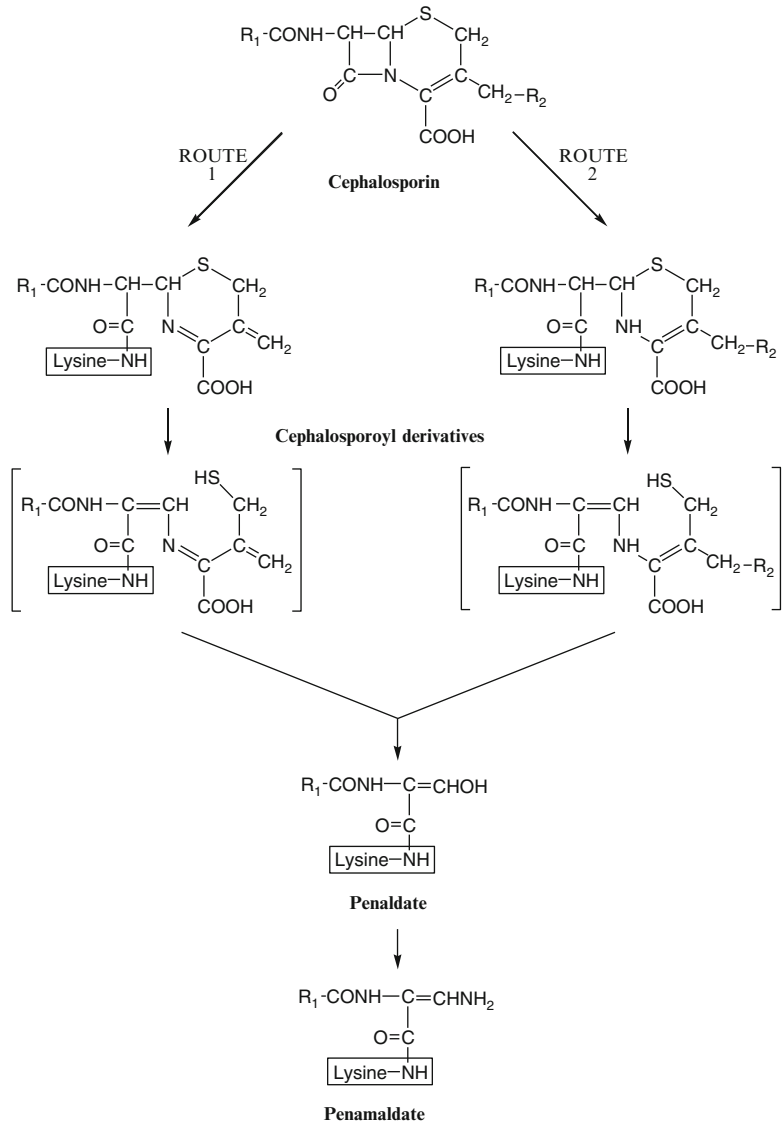


Fig. 5.14 Aminolysis of cephalosporins and decomposition products. With a good leaving group at C-3 (as in cefaclor and cephalothin), aminolysis in the presence of protein or polylysine leads to cleavage of the β-lactam ring via route 1. With a poor leaving group at C-3 (e.g., cephalexin, cefadroxil), immediate breakdown of the dihydrothiazine ring does not occur and the intact cephalosporoyl structure forms, if only temporarily, as the reaction proceeds via route 2. The unstable intermediates decompose to form the penaldate and penamaldate structures

5.2.4.1 Aminolysis of Cephalosporins and Decomposition Products

In early studies on cephalosporin C in E. P. Abraham's laboratory in Oxford, acid hydrolysis yielded no penicillamine and alkali treatment led to fragmentation of the molecule. Aminolysis of cephalosporins in the presence of polylysine or protein gave results (including the appearance of new chromophores) consistent with the formation of unstable intermediates that decompose to penaldate and ultimately penamaldate structures (Fig. 5.14). Analysis of the penamaldyl product showed that it was the amino group from the amino acid not from the nitrogen atom of the dihydrothiazine ring that was involved in the formation of the penaldate-like compound.

The nature of the leaving group at position C-3 on the dihydrothiazine ring has a significant influence on the activity of the β-lactam ring and the stability of the dihydrothiazine ring. Nucleophilic-induced β-lactam ring cleavage of cephalosporins with a good leaving group at C-3 is accompanied by elimination of the leaving group. The presence of a good leaving group as in cefaclor (Cl), cephalothin (acetoxy), and cephaloridine (pyridinium) would be expected to proceed via the formation of a cephalosproyl derivative with a double bond at Δ^4 and a methylene group at C-3 (route 1, Fig. 5.14). With a poor leaving group at C-3, for example, the methyl group in cephalexin and cefadroxil, aminolysis does not appear to cause immediate breakdown of the dihydrothiazine ring and the cephalosporoyl structure forms, if only temporarily before eventually proceeding to the penaldate and perhaps penamaldate derivatives (route 2, Fig. 5.14). In fact, even with a good leaving group such as pyridinium in cephaloridine and ceftazidime, departure of the leaving group may not be concerted with β-lactam ring opening. The rate of decomposition increases as the concentration of the compound increases and the pH decreases. Dewdney has pointed out that the still often-used cephalothin may undergo breakdown by both routes, one via direct aminolysis of the parent compound (route 1) and the other via its major metabolite deacetylcephalothin (route 2).

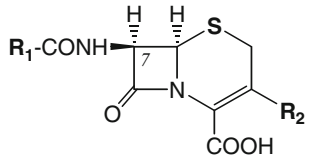
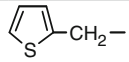
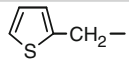
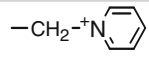
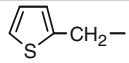
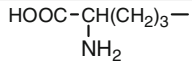
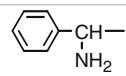
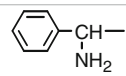
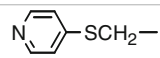
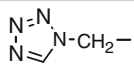
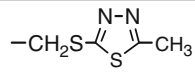
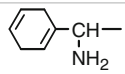
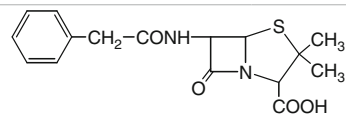
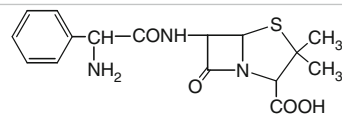
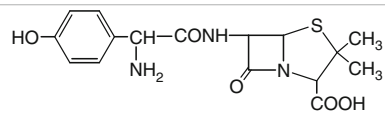
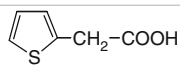
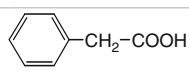
5.2.4.2 Allergenic Significance of the Acyl (R1) Side Chain of Cephalosporins

The work outlined above, and the interpretation of the chemical findings, indicates that as far as any resultant determinants are concerned, aminolysis results in structures in which only the R1 side chain, the attached amide, and remnants of the β-lactam ring remain from the original cephalosporin molecule. The resultant penaldate- and any penamaldate-like structures linked to carrier protein therefore represent hapten–carrier complexes that may interact with side chain (R1)-specific IgE antibodies in allergic responses to therapeutic dosage of cephalosporin drugs.

5.2.4.2.1 Studies Implicating the R1 Side Chain in Clinical Hypersensitivity

In the earliest demonstration of a useful in vitro immunoassay for the detection of cephalosporin-reactive IgE antibodies in the sera of patients allergic to a cephalosporin, cephalothin sodium was covalently coupled to *bis*-oxirane-activated Sepharose® (Pharmacia) (see Sect. 4.3.1) and used to detect complementary IgE in the sera of patients who experienced anaphylaxis after administration of cephalothin or cephalexin. Quantitative hapten inhibition results (Table 5.5) for one of the cephalothin-sensitive patients revealed clear recognition of the side chain of cephalothin and cross-recognition of compounds with R1 groups showing a structural similarity to the cephalothin R1 group, in particular, benzylpenicillin. This conclusion was reinforced by the significant inhibition seen with the side chain analogs 2-thiopheneacetic acid and phenylacetic acid. Bearing in mind the decomposition of the dihydrothiazine ring induced by the coupling procedure at alkaline pH, the determinant presented in the assay may be the penaldate- (see below) and/or penamaldate-like structures (Fig. 5.14), and therefore, any reactive IgE antibodies detected with the solid phase conjugate would be complementary to remaining undegraded structures, most likely the cephalothin side chain structure, and this, in fact, was found. The usefulness of the activated Sepharose radioimmunoassay for the detection of IgE antibodies to cephalosporins was also demonstrated by Romano and collaborators in assays on sera from 70 patients with immediate reactions to cephalosporins and 40 control sera from healthy, nonatopic individuals. A positive result was recorded for 52 (74.3 %) of the patients and the specificity of the assay was 100 %. Again, cross-reactions were observed and these could be explained by recognition of identical or similar side chain groups. The authors acknowledged that cephalosporin–Sepharose conjugates provide a useful diagnostic tool and could be employed as a complementary test with skin testing to evaluate cephalosporin-induced immediate reactions. Given the clear-cut demonstration of the utility

Table 5.5 Inhibition of IgE antibody binding to cephalothin-Sepharose by cephalosporins and penicillins

Compound	Structure  R_1 [b]	R_2 [b]	Inhibition (%) of binding of IgE antibodies[a] with 400 nmol of compound to cephalothin-Sepharose
Cephalothin		$-CH_2OCOCH_3$	57
Cephaloridine			58
Cefoxitin[c]		$-CH_2OCONH_2$	33
Cephalosporin C		$-CH_2OCOCH_3$	9
Cephaloglycin		$-CH_2OCOCH_3$	28
Cephalexin		$-CH_3$	0
Cephapirin		$-CH_2OCOCH_3$	26
Cefazolin			18
Cephradine		$-CH_3$	14
Benzylpenicillin			44
Ampicillin			12
Amoxicillin			4
2-Thiopheneacetic acid			31
Phenylacetic acid			20

From Harle DG, Baldo BA. Drugs as allergens: An immunoassay for detecting IgE antibodies to cephalosporins'. Int Arch Allergy Appl Immunol. 1990; 92: 439. Reprinted with permission from S. Karger AG, Basel

[a]IgE in serum from patient with anaphylaxis to cephalothin

[b]R_1 and R_2 refer to cephalosporins, not to penicillins and other compounds examined

[c]Cefoxitin is further characterized by the presence of a methoxy residue ($-OCH_3$) at position 7 of the β-lactam ring

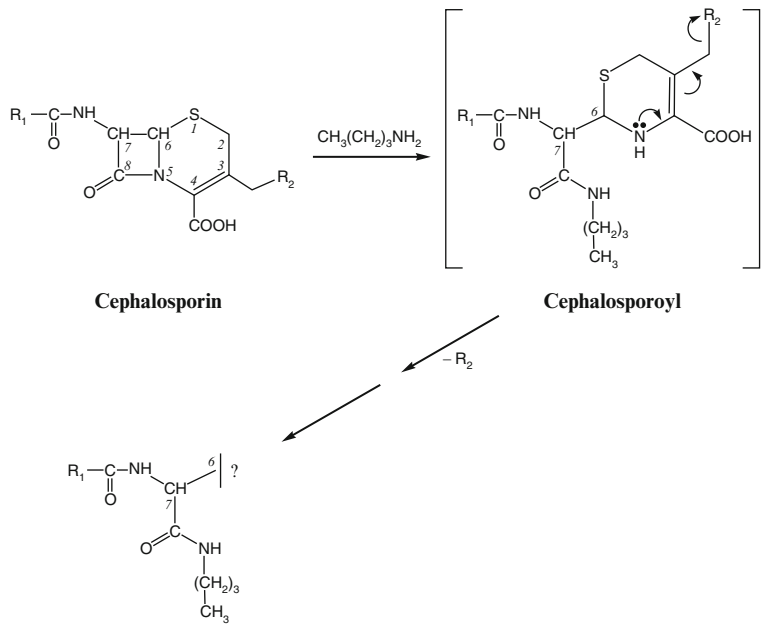


Fig. 5.15 Determination, prior to intended synthesis, of model structure with intact side chain remaining after aminolysis of cephalosporins. Adapted from Montañez MI et al. Synthetic Approach to Gain Insight into Antigenic Determinants of Cephalosporins: In vitro Studies of Chemical Structure–IgE Molecular Recognition Relationships. Chem Res Toxicol 2011; 24: 706. Adapted with permission from ©2011 American Chemical Society

and performance of the Sepharose assay, its ease of preparation, and proven capacity to detect side chain groups on cephalosporins, the method cannot be dismissed simply because the dihydrothiazine ring is lost during preparation of the drug-solid phases. The procedure has worked well to detect R1 determinants on cephalothin, cefaclor, cephalexin, ceftriaxone, cefotaxime, ceftazidime, cefonicid, cefuroxime, and cefamandole and is particularly effective when used with cephalothin and cefaclor. Because of the known disintegration of the dihydrothiazine ring, nucleophilic addition of the *bis*-oxirane-activated solid phase at alkaline pH to the S or N atom of the six-membered ring may not be possible, but it might proceed by reaction with the N of the aminobenzyl and S of the thiophene groups of the R1 side chains of cefaclor and cephalothin, respectively, although this might be expected to interfere with IgE interaction with the side chain. Efforts should be made to characterize the structures retained after coupling and if, as seems highly likely, these prove to be penaldate- and/or penamaldate-like, the procedure and test reagents should continue to be considered as useful and convenient diagnostic tools.

In a study aimed at gaining insights and preparing and ultimately utilizing antigenic determinant structures of cephalosporins, Perez-Inestrosa and collaborators set out to synthesize the proposed structure remaining on the carrier protein after the chemical breakdown resulting from aminolysis of the cephalosporin molecule. *n*-Butylamine was used as the nucleophile instead of the primary amino groups of lysine residues in proteins. The proposed aminolysis pathway and model structure to be utilized in allergenic determinant investigations are shown in Fig. 5.15. Note that the structure remaining after disintegration of the dihydrothiazine ring retains the entire R1 side chain group of the original cephalosporin so, in any diagnostic application, only side chain-specific IgE antibodies would be detected. From research in the late 1960s and early 1970s and

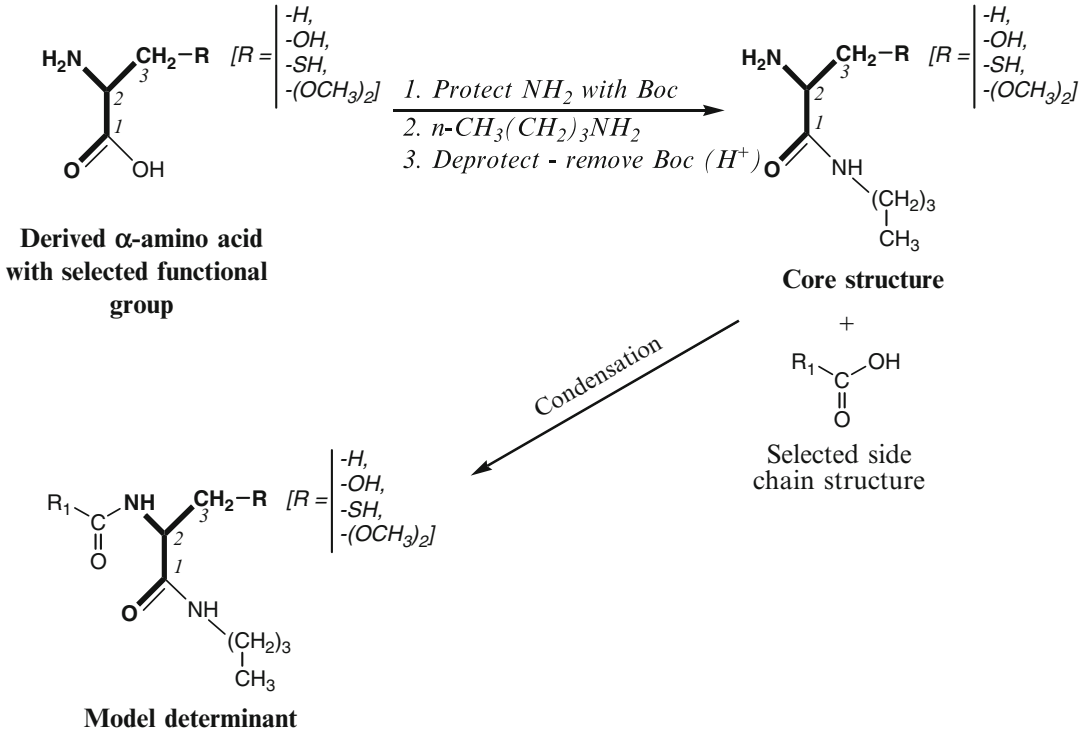


Fig. 5.16 Synthesis of the core structure (determined from products of the aminolysis pathway—see Fig. 5.15) and model haptenic structures with different R1 side chain groups and functional groups attached at C-3. From Montañez MI et al. Synthetic Approach to Gain Insight into Antigenic Determinants of Cephalosporins: In vitro Studies of Chemical Structure–IgE Molecular Recognition Relationships. Chem Res Toxicol 2011; 24: 706. Adapted with permission from ©2011 American Chemical Society

NMR data, it can be inferred that the model determinant has a penaldate-like structure. Based on knowledge of the aminolysis pathway and the likely structure of this determinant, a synthesis was devised for a series of antigenic determinants with the same core structure (shown in bold, Fig. 5.16) but with differences in the R1 group and the functional group at C-3 [C-6 in the original, undegraded cephalosporin molecule (Fig. 5.15)]. Since the nature of the substituent at C-3 is unknown, and this C atom in the intact parent molecule (attached to a S atom) was capable of undergoing a number of different reactions including reactions with O and S, hydroxyl, thiol, and acetal groups were investigated as functional groups at C-3. For the preparation of a methyl group at C-3, an alanine derivative can be used as the starting derived α-amino acid, and for the OH and SH functional groups, the desired serine and cysteine derivatives, respectively, are utilized. When these model determinants were used in hapten inhibition experiments with sera from cephalosporin-allergic patients and cephalosporin-solid phases, optimal inhibition was seen with model structures containing the R1 side chain of the cephalosporin that induced the reaction. Molecules containing OH and $O(CH_3)_2$ at C-3 were slightly better inhibitors, suggesting to the investigators that these structures mimic the real antigenic determinant involved in the initial allergic reaction. By contrast, the presence of an S atom at C-3 drastically decreased inhibition, often abolishing it and suggesting that, in vivo, cephalosporin antigenic determinants may occur via breaking of the S-1–C-6 bond. A puzzling feature of this work and its strategy is the nature of the cephalosporin conjugates used on the solid phases for the testing of the inhibitory activities of the model determinants. The preparations of the cephalosporin–poly-L-lysine conjugates used

in the radioimmunoassay were not described, but the references provided are for the preparation and examination of benzylpenicilloyl and amoxicilloyl conjugates, suggesting that the equivalent conditions and procedures were employed for the preparation of the cephalosporin conjugates. If that was not the case and conditions leading to disintegration of the dihydrothiazine ring were not employed, the nature of the different cephalosporin test reagents used remains unclear. After devising a strategy to avoid the lability of the cephalosporins and undertaking chemical syntheses to produce and precisely define the model determinants, the same thinking and approach are needed for the preparation of the cephalosporin-solid phases if the studies' conclusions are to be beyond criticism.

There have been a number of reports of anaphylaxis and other immediate reactions to cephalosporins with a 2-aminothiazol-4-yl R1 group seen, for example, in reactions to ceftriaxone, ceftazidime, cefepime, cefodizime, and cefixime with cross-sensitivity between these drugs demonstrated in skin tests. Some patients with immediate clinical and skin test reactions to cefuroxime, which has a 2-furyl substituent in the R1 side chain, were also shown to be skin test positive to cephalosporins with the 2-aminothiazolyl R1 group. The chemical basis for this cross-recognition appears to be a common alkoxyimino substituent in the R1 groups. This can be concluded from positive skin test reactions to cefuroxime, cefotaxime, cefepime, ceftriaxone, and oxacillin in a patient who experienced anaphylactic shock after receiving cefuroxime axetil. The first four of these compounds have a methoxyimino group while oxacillin has a bioisostere of the methoxyimino in the form of an isoxazolyl group with a methyl substituent (Fig. 5.17).

5.2.4.2.2 In Vitro and In Vivo Cross-Reactivity Between Cephalothin and Benzylpenicillin

Antigenicity of the R1 side chain group has been amply demonstrated by examination of the specificities of antisera from laboratory animals immunized with cephalosporin–protein conjugates. Early studies on rabbit antibodies to cephalothin and benzylpenicillin demonstrated cross-reaction between the two drugs and this cross-reactivity was confirmed in humans by a number of anaphylactic reactions to cephalothin in penicillin-allergic patients on first exposure to the cephalosporin. In addition, positive intradermal tests to cephalothin were seen in patients with known allergic reactions to penicillins, but previously unexposed to cephalothin and passive transfer tests with sera from cephalosporin-allergic and penicillin-allergic patients gave positive reactions to challenges with benzylpenicillin and cephalothin, respectively. Cross-reaction between the two β-lactams was also suggested by results of IgE antibody direct binding studies in the authors' laboratory where the frequencies of detection of benzylpenicillin- and cephalothin-reactive IgE antibodies in 1,797 routine tests on sera from patients with suspected allergy to a penicillin(s) and/or a cephalosporin(s) were compared. Sera from 123 patients (6.8 %) were positive to both benzylpenicillin and cephalothin, 238 sera (13.2 %) proved positive to cephalothin and negative to benzylpenicillin, and only 15 sera (0.8 %) were positive to the penicillin but negative to the cephalosporin. Inhibition experiments with rabbit antisera had implicated the side chains as the source of the cross-recognition and this conclusion was supported by results of quantitative hapten inhibition experiments with patients' sera containing IgE antibodies which clearly revealed that cross-recognition is due to the side chain (R1) groups on each molecule with the methylene group as the focus of the allergenic determinant and with the probable contribution of the bioisosteric benzene and thiophene rings. The exact confines of the determinant at the β-lactam ring end of the side chain could not be determined, but inhibition findings with some analogs suggested that it probably makes some contribution. Interestingly, both direct binding and inhibition investigations showed that the cross-reactive IgE antibodies recognized benzylpenicillin more strongly than cephalothin and this may be relevant to the observation that many patients allergic to penicillin have tolerated cephalothin.

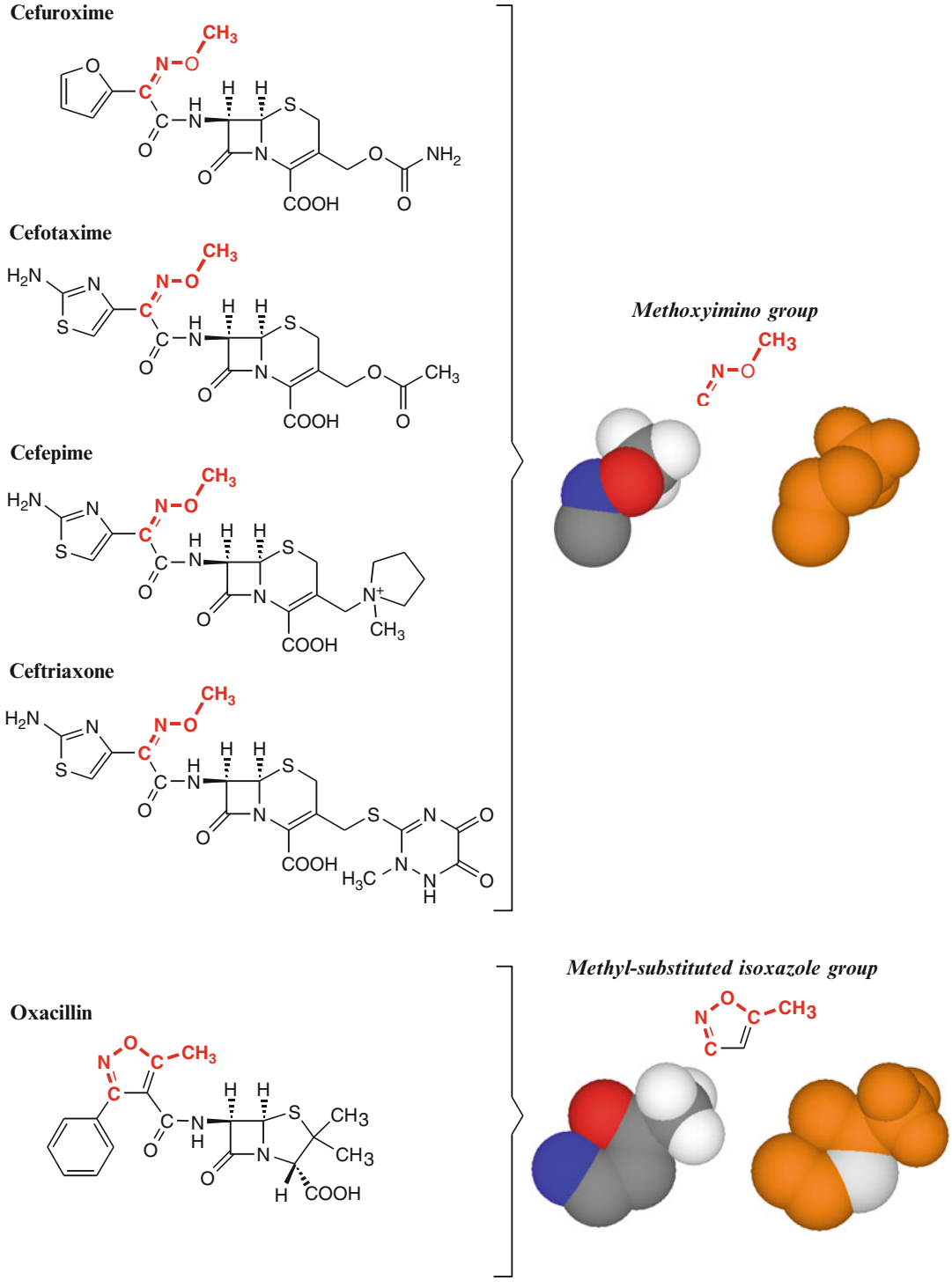


Fig. 5.17 Cephalosporins with a methoxyimino group in the R1 side chain responsible for skin test reactivity in a patient who experienced anaphylaxis to cefuroxime axetil. Oxacillin, which contains a methyl-substituted isoxazolyl group, a bioisostere of the methoxyimino group, was also skin test-positive in the patient. Two-dimensional structures of the four cross-reacting cephalosporins and oxacillin are shown with the methoxyimino groups of the former and the methyl-substituted isoxazole of the latter highlighted in *red*. Three-dimensional models of these proposed cross-reactive groupings of atoms are also shown with the related sequence of atoms C=N–O–CH_3 (in the cephalosporins) and C=N–O–C–CH_3 (oxacillin) highlighted in *orange*. (Results from Hasdenteufel F et al. Ann Pharmacother 2007; 41: 1069)

5.2.4.3 Summary of the Current Situation with Cephalosporin Determinant Structures: Are R2 Side Chains Recognized as Allergenic Determinants?

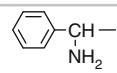
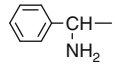
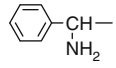
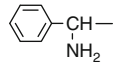
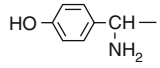
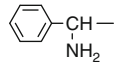
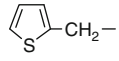
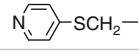
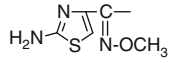
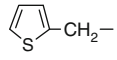
For many years, knowledge of cephalosporin determinant structures and diagnostic application of such determinants has not matched the chemical insights and clinical applications that began 50 years ago with the structurally related β-lactams, the penicillins. In recent years, a paradoxical situation has existed with the cephalosporins—IgE antibody tests for the specific detection of cephalosporin allergenic determinants have proved to be effective assays and useful diagnostically, but the chemical understanding of the drug conjugates and solid phases employed as test reagents has been lacking. Some recent chemical work discussed above has begun to improve this situation, but doubts remain about the apparent belief that, because of the lability of the dihydrothiazine ring, the R1 side chain group is the only relevant allergenic structure of the cephalosporin molecule.

5.2.4.3.1 Recognition of the R2 Side Chain

Although the antigenic preeminence of the R1 side chain may ultimately prove to be correct, there is already evidence that with some cephalosporin-allergic patients, in addition to recognition of the R1 structure, the R2 side chain and/or the whole cephalosporin molecule are recognized by serum IgE antibodies and this may be by one or more populations of antibodies. This can be seen by analysis of the quantitative inhibition data selected from results of a study of fine structural recognition patterns demonstrated by cephalosporin-reactive IgE antibodies in the sera of cephalosporin-allergic patients (Table 5.6). In an attempt to avoid the uncertainties resulting from opening the β-lactam ring and the associated dihydrothiazine ring fragmentation, cefaclor, cephalexin, and cephalothin solid phases for use in radioimmunoassays to detect cephalosporin-reactive IgE antibodies were prepared by linking HSA to the carboxyl group at position 4 with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC). Excellent uptakes of IgE antibodies in patients' sera by the cephalosporin-HSA solid phases were seen in direct binding assays and specificity was demonstrated by clear inhibition of binding after preincubation of the sera with free cephalosporin drugs but not with penicillins (benzylpenicillin, ampicillin, amoxicillin, and becampicillin), aztreonam (a monobactam), and clavulanic acid (a clavam). Imipenem (a carbapenem) and moxalactam (an oxacephem) showed weak inhibitory activity with a few sera. From the inhibition results, it became clear that although the R1 group was the dominant specificity, both side chains contributed to the IgE antibody recognition profiles as shown by some results summarized in Table 5.6 and interpreted in Table 5.7. In particular, with serum 3,252, a comparison of the inhibitory activities of cefaclor on the one hand and cephalexin and cephaloglycin on the other demonstrated that the aminobenzyl group at R1 and Cl at R2 were the preferred structures for interaction with the cefaclor-reactive antibodies. These results suggested that the complementary IgE antibodies recognized the whole cefaclor molecule. Recognition of the ester group at R2, demonstrated by strong inhibition with cephaloglycin and cephalothin, was seen with serum 3,323 and this, together with a clear requirement for the aminobenzyl group at R1 and moderate inhibition by loracarbef which has a Cl at R2, suggested the possible presence of a second, cross-reactive population of IgE antibodies. The strong requirement for the aminobenzyl group at R1 for serum 4,679, together with clear-cut recognition of the ester group at R2 (demonstrated by the strong inhibition with cephaloglycin), again suggested the possible recognition of a second allergenic specificity. With serum 4,764, recognition of the aminobenzyl group at R1 was accompanied by a requirement for a "small" group (Cl or CH_3) at R2.

Apart from findings from the authors' laboratory, some other observations made during the investigation of patients' immediate reactions to cephalosporins suggest that specificities in addition to, or other than, the R1 group are recognized by IgE antibodies. In inhibition studies undertaken in Spain with the sera of three patients

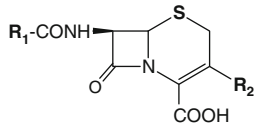
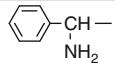
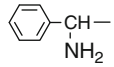
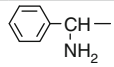
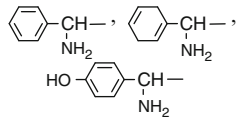
Table 5.6 Inhibition by cephalosporins of binding of IgE antibodies in the sera of cephalosporin-allergic patients to cefaclor–HSA[a]. Recognition patterns indicating drug IgE-binding determinants[b]

Cephalosporin inhibitor	Structure R_1	R_2	Inhibition (%) of IgE antibody binding by 500 nmol of cephalosporin with serum 3,252	3,323	4,679	4,764
Cefaclor		–Cl	82	95	77	92
Loracarbef (*a carbacephem*)		–Cl	58	33	35	91
Cephalexin		$-CH_3$	32	6	52	89
Cephradine		$-CH_3$	31	21	22	88
Cefadroxil		$-CH_3$	12	4	8	88
Cephaloglycin		$-CH_2OCOCH_3$	10	69	88	9
Cephalothin		$-CH_2OCOCH_3$	5	57	11	3
Cephapirin		$-CH_2OCOCH_3$	7	0	0	0
Cefotaxime		$-CH_2OCOCH_3$	11	14	3	2
Cefoxitin (*a cephamycin*)		$-CH_2OCONH_2$	0	14	5	11

[a]Prepared by coupling human serum albumin to the 4-carboxyl group of cefaclor
[b]Refer to Table 5.7 for interpretation
From Pham NH, Baldo BA. β-Lactam drug allergens: Fine structural recognition patterns of cephalosporin-reactive IgE antibodies. J Mol Recognition 1996;9:287. Reprinted with permission from John Wiley and Sons

allergic to cefaclor, inhibition was almost exclusively confined to cefaclor while no inhibition was seen with ampicillin even though this penicillin has the same R1 group as cefaclor. This was explained by the suggestion that the allergenic specificity complementary to the IgE antibodies was the R1 group "plus the remaining cephalosporin structure." Given the absence of inhibition by ampicillin, it is not clear why the R1 group was still assumed to be central to recognition nor was the "remaining cephalosporin structure" explained further. From test results on a number of different individual patients, two other clear examples difficult to reconcile with the view that allergic sensitivity to a cephalosporin occurs exclusively via recognition of the R1 side chain are mentioned. In an Italian study, a patient who had anaphylaxis provoked by cefodizime and who was skin test positive to the drug was found to be skin test negative to two other cephalosporins with the same R1 group (ceftriaxone and cefotaxime) and to two other cephalosporins with different but structurally closely related R1 groups (ceftazidime and cefuroxime). In addition, challenges with ceftriaxone and cefuroxime were negative. The most likely explanation seems to be allergic recognition of the entire cefodizime molecule (as previously demonstrated

Table 5.7 Recognition by IgE of R_1 and R_2 side chains on cephalosporins and interpretation of recognition patterns (refer Table 5.6)

	Recognition at R_1 and R_2		
			
Serum	R_1	R_2	Important features of inhibition findings
3,252		$-Cl \gg -CH_3$	Entire molecule recognized. Little tolerance for change at R_1 (aminobenzyl) and R_2 (–Cl) (c.f. inhibitions with cefaclor and cephalexin)[a]
3,323		$-Cl > -CH_2OCOCH_3 > -CH_3$	Requirement for aminobenzyl at R_1 and –Cl at R_2 but with some clear recognition of ester group—presence of a second cross-reactive (with cefaclor) IgE with some tolerance at R_1 and ester at R_2? (Note inhibitions with cephaloglycin and cephalothin)[a]
4,679		$-CH_2OCOCH_3 > -Cl \gg -CH_3$	Strong requirement of aminobenzyl at R_1 but with tolerance at R_2. Inhibition with cephaloglycin[a] may indicate – Preference for ester at R_2 – Or a second allergenic specificity
4,764		$-Cl = -CH_3$	Requirement for aminobenzyl or closely related structure at R_1 and a "small" group at R_2 (–Cl *or* $-CH_3$)

From Pham NH, Baldo BA. β-Lactam drug allergens: Fine structural recognition patterns of cephalosporin-reactive IgE antibodies. J Mol Recognition 1996;9:287. Reprinted with permission from John Wiley and Sons
[a]See Table 5.6

with cefaclor) or recognition of the R2 side chain of cefodizime which differs from the other cephalosporins tested. In findings from the same group, a patient who experienced an anaphylactic reaction to the third-generation cephalosporin cefoperazone was skin test positive to this drug and to the second-generation cefamandole. These two drugs have a structurally unrelated R1 side chain but an identical 1-methyltetrazol-5-yl-sulfonyl R2 group (Fig. 5.13).

Therefore, although it is clear that the R1 side chain is considered to be a dominant (perhaps *the* dominant) allergenic specificity found on cephalosporins, two factors may have influenced and reinforced this belief to the exclusion of any consideration of the contribution of other possible allergenic structures. The first is the known lability of the six-membered ring with the associated belief that R2 groups are readily lost both in vitro and probably in vivo and therefore the R2 side chain cannot contribute to the molecules allergenicity. The second factor, stemming directly from the first, is the nature of the structures prepared for the detection of cephalosporin-reactive IgE antibodies. These structures tend to comprise essentially the R1 side chain only. Unvalidated diagnostic reagents arising from research and discussed above are examples of the bias of tests for detection of the R1 specificity and it remains to be seen if efforts to couple cephalosporins via

the 4-carboxyl group retain stable complexes suitable for identifying potential allergenic specificities on the whole cephalosporin molecule and, in particular, on the R2 side chain.

5.2.4.3.2 Does the Spectrum of Anti-Cephalosporin IgE Antibodies Reflect the Full Range of Cephalosporin Antigens?

As discussed earlier (Sect. 5.1.3), the heterogeneity of penicillin determinants extends to the spectrum of serum IgE antibodies, so rather than trying to identify likely allergenic metabolites or breakdown products, preparing them in the laboratory, and then testing them, a more comprehensive and efficient approach might be to identify immunochemically the full spectrum of determinants recognized by a large population of cephalosporin-allergic patients. For drugs such as the cephalosporins where questions concerning the stability and existence of some structures remain, assessment of the complete allergenic recognition profile of the cephalosporin-allergic population seems even more logical. At present it is clear that the relatively mild procedures that lead to opening of the β-lactam ring generally also induce disintegration of the dihydrothiazine ring, but it is not always clear how precisely concerted this process is, whether ring breakdown occurs with a variety of other procedures, and what is the nature and range of structures patients are exposed to in vivo following ingestion or injection of a cephalosporin. Since immediate, type I allergic reactions are mediated by IgE antibodies, the full spectrum of cephalosporin allergenic determinants could, in the first instance, be obtained by using both direct antibody binding and quantitative hapten inhibition procedures. With a cephalosporin conjugate that retains the structural identity of the free drug, clear inhibitions obtained with free, unmodified drugs and carefully selected structural analogs, both free of the possibility of structural lability, would be reliable indicators of the structures that are complementary to the combining sites of the drug-reactive antibodies. For now, the obstacle to progress with this approach is the absence of suitable stable and characterized cephalosporin conjugates and drug-solid phases with the cephalosporin molecule undegraded and intact. Of course, the presence of IgE antibodies to a particular structure does not always accurately reflect allergic sensitivity to that structure but, conversely, the absence of IgE (confirmed by in vitro and in vivo techniques that are sufficiently sensitive) is invariably an indication of the absence of type I allergy. By combining information obtained from the identification of the heterogeneous spectrum of IgE antibodies complementary to determinants identified on the complete and intact cephalosporin molecule with chemical analyses and, most importantly, skin testing and perhaps challenge procedures, it may be possible to more quickly and accurately define the range of the most important cephalosporin allergenic structures. One factor that might work against this proposed strategy is the possibility that the immune response is directed to a metabolite, breakdown product, or protein adduct not detected by the employment of free, unmodified drug. This, however, is likely to be anticipated and unlikely to nullify the potential success of the strategy but, in any case, no cephalosporin has yet been found to form a hapten–protein adduct without laboratory intervention.

5.2.5 Skin Testing with Cephalosporins

Skin testing for allergic sensitivity to cephalosporins has not been straightforward for at least three main reasons—the lack of standardized and characterized cephalosporin determinants of known composition and structure, the employment of concentrations of free drug that elicit irritant reactions, and the widespread impression that skin testing with these drugs produces unreliable results. Concentrations of cephalosporins up to as high as 250 mg/ml have been used intradermally and although concentrations up to 50 mg/ml of some cephalosporins were nonirritant in control subjects, a concentration of 2 mg/ml for both prick and intradermal testing seems to be satisfactory in terms of sensitivity and specificity. Using this concentration, Romano performed prick and intradermal skin tests on

76 patients with histories of immediate reactions to cephalosporins. Individual cephalosporins tested were cephalothin, cefamandole, ceftazidime, ceftriaxone, cefuroxime, and cefotaxime. Positive responses were obtained in 60 of the patients (78.9 %) and the authors concluded that skin testing at a cephalosporin concentration of 2 mg/ml is a useful tool for evaluating immediate reactions to this β-lactam.

Test solutions should be freshly prepared, preferably using solutions of drugs for intravenous injection and with all manipulations carried out under sterile conditions using aseptic precautions. If powdered drug is used in pure form or from capsules or tablets, solutions in sterile physiological saline should be prepared under sterile conditions and passed through a bacterial filter. In the prick test, read after 15–20 min, a wheal greater than 3 mm in diameter accompanied by erythema together with a negative response to a saline control is considered positive. In intradermal testing, an increase in diameter larger than 3 mm is considered positive. Any increase is obtained by comparing the marked diameter of the wheal at 20 min with the marked diameter of the initial injection bleb. As usual for skin testing, a histamine-positive control should be included. An important precaution to observe in testing for immediate reactions to cephalosporins is the need to keep in mind the patient's last exposure to the drug since the longer that exposure, the greater the chance the test will be negative. In fact, Romano has suggested that cephalosporin IgE-mediated hypersensitivity may be a transient condition. Some patients have been shown to test negative 1 year after an allergic reaction to penicillins and the same may be true for cephalosporins so a negative skin test result may not be reliable. This also raises the questions of whether challenge tests or a repeat skin test should be undertaken and, if so, is resensitization of the patient a possibility?

Comment should be made on what sometimes seems like a preoccupation with employing penicillins in skin tests to predict allergy to cephalosporins. Investigations have shown that <20 % of patients with immediate reactions to cephalosporins react to benzylpenicillin, amoxicillin, and ampicillin, suggesting that testing with penicillins does not reliably predict cephalosporin allergy unless the side chains of the penicillin and the culprit cephalosporin are similar. In other words, if the side chain of the penicillin is identical or similar, skin testing with a penicillin *may* be of value, but skin test and IgE antibody results have shown that about 90 % of patients with benzylpenicillin or amoxicillin allergy tolerate cephalosporins even when the side chains (i.e., the R1 cephalosporin side chain) on the two classes of drugs are the same or similar. Given that skin testing with cephalosporins at nonirritant concentrations appears to be an effective diagnostic procedure, there seems little logic in employing penicillins for the purpose. Some patients respond with a positive skin test only to the causative cephalosporin and this is probably to be expected given the findings that the whole cephalosporin molecule or some individual structural features have been identified as allergenic determinants (see Sect. 5.2.4.3). In these patients, negative skin test results with other cephalosporins appear to be a reliable indicator of tolerance to those cephalosporins, although more investigations of this important observation are needed.

5.2.6 Delayed Reactions to Cephalosporins

Like penicillins, cephalosporins can cause delayed hypersensitivity reactions manifesting mainly as maculopapular or morbilliform rashes and delayed urticaria/angioedema. Intradermal tests with delayed readings and/or patch tests have been recommended for diagnosis and prick testing has also been used but less often and with less success. In one study of 290 patients with cutaneous adverse reactions to drugs, cephalosporins elicited positive patch tests in 4.1 % of the patients. A recent study is summarized to illustrate the diagnostic application of skin, patch, and provocation tests in the diagnosis of delayed reactions to cephalosporins. Skin tests were undertaken with cefaclor, cephalexin, and cefatrizine at concentrations of 2 and 20 mg/ml, the latter concentration having been found to be nonirritant in

30 controls. Late readings of intradermal tests were taken at 48 and 72 h. For patch tests, cephalosporins were used at 5 % w/w in petrolatum, occlusion time was 48 h, and readings were made 24 h later, 15 min after removing the strips. Patients who gave negative results with skin and patch tests were challenged with the causative drug. Oral doses used were: cefaclor 500 mg, cephalexin 1 g, cefatrizine 500 mg, cefixime 400 mg, cefuroxime axetil 500 mg, cefprozil 500 mg, cefpodoxime 200 mg, and ceftibuten 400 mg, while 1 g of cefamandole, cefazolin, ceftriaxone, ceftazidime, cefotaxime, cefepime, and cefonicid were each administered intramuscularly. Initial challenge doses were one-hundredth of the therapeutic dose. One week later, negative responders were given one-tenth of the normal dose and, if the response was again negative, a full dose was administered 1 week later. Of 105 patients, only five (4.7 %) had delayed reactions with intradermal tests positive in the five patients and patch tests positive in three. The investigators concluded that intradermal tests are useful for identifying the cephalosporin responsible for delayed reactions and that patch tests are not recommended for cephalosporin-induced maculopapular and urticarial rashes.

5.3 Monobactams

These monocyclic drugs contain only the ring structure that gives the family of β-lactam antibiotics their name. The prototype drug of the group is the synthetic compound *aztreonam* which has the distinctive features of a sulfate group on the nitrogen of the β-lactam ring and a thiazolyl group in the side chain (Fig. 5.18a). In initial studies to assess whether aztreonam cross-reacted with other β-lactam antibiotics, Adkinson and colleagues used penicilloyl-, cephalosporoyl-, and aztreonyl-protein conjugates in solid-phase radioimmunoassay inhibition experiments with rabbit antibodies to the benzylpenicilloyl, cephalosporoyl, and aztreonyl determinates. The results clearly demonstrated that aztreonam showed little, if any, cross-reaction, indicating that the β-lactam nucleus was not being recognized and it was predicted that the side chain of the drug, rather than the core, would be the main immunogenic site. This prediction was confirmed when ceftazidime, a cephalosporin with a side chain identical to aztreonam, completely inhibited the rabbit anti-aztreonyl antibodies. Further evidence for the lack of cross-reactivity with

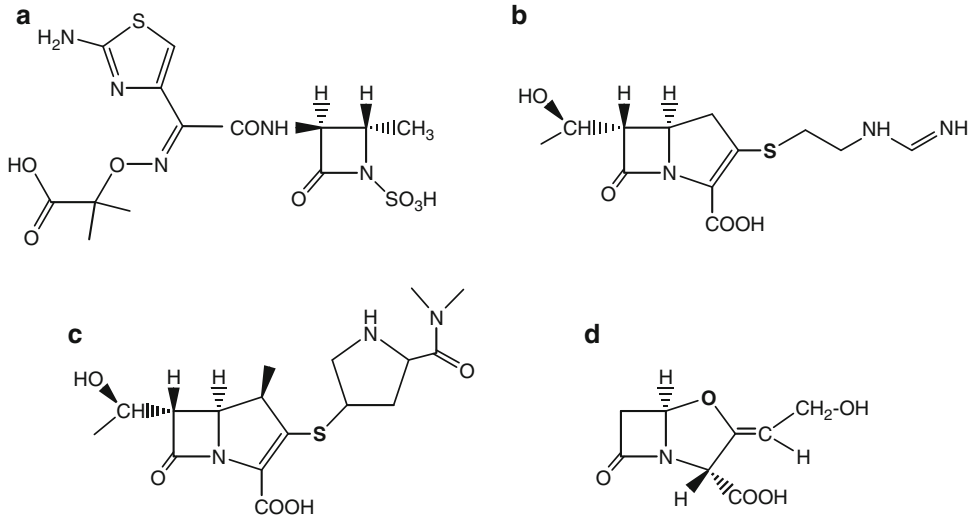


Fig. 5.18 Structures of the monobactam aztreonam (**a**), the carbapenems, imipenem (**b**) and meropenem (**c**), and the clavam, clavulanic acid (**d**). Note that clavams have an oxazolidine ring instead of the thiazolodine ring found in penicillins

penicillin determinants was seen in the failure of aztreonam–protein conjugates and free drug to react with human anti-benzylpenicilloyl IgE antibodies and in negative skin tests to aztreonam major and minor determinants (analogous to these determinants in penicillin) in patients who were skin test positive to penicillins.

In general, aztreonam is well tolerated in patients allergic to other β-lactam antibiotics, but usage has shown that aztreonam occasionally causes IgE antibody-mediated reactions including urticaria, angioedema, and anaphylaxis and this may occur on first exposure. Other adverse reactions include rashes, erythema multiforme, eosinophilia, and gastrointestinal effects. Sensitization to aztreonam and its cross-reactivity with other β-lactams were assessed in high-risk patients with cystic fibrosis where it was well tolerated, but anaphylactic reactions in two patients on reexposure to the drug and a 5 % skin test cross-reactivity with other β-lactam antibiotics suggested that caution should be exercised in administering aztreonam to cystic fibrosis patients allergic to other β-lactam antibiotics because it is potentially allergenic on repeated use. A retrospective study of hypersensitivity reactions to aztreonam and other β-lactams in cystic fibrosis patients revealed a wide incidence of reactions ranging from 50.9 % for piperacillin to 4 and 6.5 % to imipenem and aztreonam, respectively. There was no discernable cross-reactivity and the reactions to aztreonam seemed to be restricted to a small group of reactors with a high propensity for hypersensitivity to β-lactams. A cross-reaction due to the identical side chains on aztreonam and ceftazidime was detected by positive skin tests to both drugs in a patient allergic to aztreonam but skin test and challenge test negative to a number of penicillins and other cephalosporins. By contrast, four cystic fibrosis patients who were allergic to ceftazidime demonstrated tolerance to aztreonam.

5.4 Carbapenems

In addition to the β-lactam ring, the carbapenem nucleus contains an unsaturated five-membered ring as for penicillins but with the sulfur atom of the latter replaced by a carbon atom. The two most used drugs from this group are the broad spectrum antibiotics *imipenem* (Fig. 5.18b) and *meropenem* (Fig. 5.18c). Imipenem is formulated with cilastatin, a compound without antibacterial activity but a specific inhibitor of renal dihydropeptidase 1, the enzyme responsible for the metabolism of imipenem and all other naturally occurring carbapenems. Meropenem is stable to the enzyme and is administered without cilastatin. Imipenem, especially in minor determinant form, cross-reacts with penicillins and should therefore be regarded with caution before administering it to patients with suspected penicillin allergy. From the initial investigations by Saxon and colleagues, the frequency of cross-reactivity between imipenem and penicillins as defined by skin testing was about 47 % and this dropped to 25 % for subjects reporting a penicillin allergy, that is, those with positive *and* negative skin test results. Recent retrospective investigations claim the figure is about 9–11 %. In the general population, the frequency of hypersensitivity to carbapenems has been estimated as less than 3 %. For patients with delayed-type hypersensitivity to penicillins, cross-reactivity between imipenem and other β-lactams studied by delayed reading intradermal and patch tests is said to be 5.5 %.

Results from more recent prospective studies have continued to show that the formerly accepted perception of risk of administering carbapenems to penicillin-allergic subjects was probably an overestimation. Skin testing with imipenem (0.5 mg/ml) of over 100 patients each with at least one positive skin test to a penicillin reagent revealed only one positive reactor. Intramuscular challenges with increasing concentrations of imipenem–cilastatin over a 3 h period showed no reactors in the skin test-negative group. The 0.9 % incidence of positive reactions found is significantly lower than previous estimates and lower than the 4.4 % rate of cross-reactivity claimed for penicillins and cephalosporins. The results bring into question the practice of avoiding imipenem in penicillin-allergic patients and indicate that prophylactic skin tests can be useful in patients who require treatment with imipenem–cilastatin. If the negative predictive value of skin testing with imipenem is in doubt, patients

allergic to penicillins and skin test negative to imipenem can be subjected to graded challenge with the drug. Similar studies on meropenem and imipenem in children led to similar findings and conclusions. Skin testing and challenges showed that reactivity to meropenem at 1 mg/ml and penicillins in patients with IgE-mediated allergy to penicillins was no higher than 5.2 % and the data indicated that no more than 3.5 % of patients would show a positive challenge after a negative skin test. On the basis of these findings, it was suggested that the practice of avoiding meropenem therapy in penicillin-allergic patients should be reconsidered. Given the much lower frequency of cross-reactivity found in this study, it may be significant that only the parent carbapenem was employed for skin testing whereas in the earlier investigation by Saxon, imipenem metabolites were also used. A second proviso to be kept in mind is that after challenges, patients did not receive a full therapeutic course of carbapenem. The absence of allergic cross-reactions of carbapenems with penicillins has also been deduced following conclusions formed from extensive clinical experience with several hundreds patients over a 12-year period. Little or no cross-reactivity was seen even in patients with a history of anaphylaxis to penicillins and who were not skin tested.

The question of tolerability of penicillins, monobactams, and carbapenems in patients with IgE hypersensitivity to cephalosporins was recently assessed in 98 subjects by serum IgE antibody assays, challenge tests, and skin testing with penicillin reagents, aztreonam, imipenem–cilastatin, and meropenem. Approximately 25 % of cephalosporin-allergic subjects were positive to penicillins, while 3.1, 2, and 1 % showed positive results to aztreonam, imipenem, and meropenem, respectively. A reaction to a cephalosporin with a similar or identical side chain to penicillin was a significant predictor of cross-reactivity. For skin testing, the following concentrations were used: ampicillin and amoxicillin 1 and 20 mg/ml; cephalosporins 2 mg/ml; aztreonam 2 mg/ml; imipenem–cilastatin 0.5 mg/ml; and meropenem 1 mg/ml.

From the relatively few investigations carried out on the carbapenems, it seems clear that potentially cross-reactive IgE antibodies to the β-lactam ring either do not occur or are rare in patients allergic to penicillins. It therefore seems likely that IgE antibodies to imipenem and meropenem generally recognize the groups attached at C-6 and to the sulfur atom at C-3 of the bicyclic nuclear structure.

5.5 Clavams

Like penicillins, *clavulanic acid* is a bicyclic structure with a β-lactam ring but, unlike penicillins, it lacks an R1 side chain and has an oxazolidine ring instead of the thiazolidine ring (Fig. 5.18d). The compound has negligible antimicrobial activity but binds to the active site of β-lactamase ultimately inactivating the enzyme. It is therefore sometimes formulated with penicillins such as amoxicillin and ticarcillin to prevent inactivation of the antibiotic and retain antimicrobial action. Early investigations showed that the compound was poorly immunogenic and, until fairly recently, it was generally assumed that it demonstrated low or no allergenicity. Over the last few years, there seems to have been an increase in cases of immediate hypersensitivity to clavulanic acid, leading to speculation that this is a reflection of its increasing usage with amoxicillin. The presence of the antibiotic in the combination, and the limited availability and stability of the enzyme inhibitor, has hampered ready diagnosis of hypersensitivity to clavulanic acid. To overcome its lability, freshly preserved solutions should be prepared for testing and cellulose or silica-based excipients may be added. For skin prick testing, clavulanic acid and excipient each at 10 mg/ml have been used, and for intradermal tests, the concentration is reduced to 1 and 0.1 mg/ml.

In an evaluation of 276 patients who had a reaction attributed to amoxicillin–clavulanic acid, 55 patients (19.9 %) reacted positively to different penicillin determinants. Of the 221 with negative skin tests, 15 were positive to amoxicillin and seven were judged to be allergic to clavulanic acid on the basis of tolerance to benzylpenicillin and amoxicillin and an immediate reaction to clavulanic acid following challenges. In the skin test-positive group, skin and

challenge tests showed negative reactions to benzylpenicillin reagents and amoxicillin but positive skin tests to clavulanic acid in 16 patients. For skin testing, clavulanic acid was used at a concentration of 20 mg/ml and amoxicillin–clavulanic acid at 20 mg/ml amoxicillin and 4 mg/ml clavulanic acid. This difference in concentrations between test solutions of clavulanic acid used alone or in combination with amoxicillin may explain why more positive skin test reactions to the clavam have not been detected. Even so, the finding that 29 % of immediate allergic reactions to the amoxicillin–clavulanic combination are directed to clavulanic acid is surprisingly high. As well as skin tests, detection of CD63 expression by basophils in the basophil activation test using flow cytometry and sulfidoleukotriene release by basophils in the cellular antigen stimulation test (CAST) have been used to demonstrate positive allergic responses to amoxicillin–clavulanic acid and clavulanic acid alone and negative responses to amoxicillin alone and other β-lactam antibiotics.

Hepatitis and jaundice caused by the combination of amoxicillin and clavulanic acid were first identified in 1988. The combination of the two drugs is associated with a higher incidence of liver injury than the administration of amoxicillin alone. The risk of this drug-induced liver injury, mostly cholestatic in nature, increases with age and by about a factor of 3 after 2 or more consecutive courses of drug.

There are at least three reports of delayed reactions to clavulanic acid manifesting as generalized urticaria, generalized itchy erythema, and allergic contact dermatitis with pruritic erythematous patches. Diagnoses were confirmed by patch testing with amoxicillin–clavulanic acid dried syrup at 10 and 1 mg/ml and clavulanic acid 10 mg/ml and for one patient, by skin prick and intradermal tests.

Summary

- The β-lactam antibiotics comprise four main classes of drugs that possess antibacterial action, viz., penams (penicillins), cephems (cephalosporins), monobactams, and carbapenems.
- Penicillins have long been known to be the most common cause of drug-induced allergic reactions including anaphylaxis.
- Penicillins can cause all four types of hypersensitivity responses provoking type I IgE-mediated reactions such as urticaria, angioedema, asthma, and anaphylaxis; type II antibody-mediated hemolytic anemia and thrombocytopenia; type III immune complex-mediated serum sickness-like reactions and vasculitis; and type IV T cell-mediated contact dermatitis, rashes, and other skin eruptions.
- Investigations of the formation of antigenic and allergenic determinants of benzylpenicillin have identified a list of determinants that includes the penicilloyl, penicillenate, penicilloic acid, penilloic acid, penamaldate, penicoyl, penicillanyl, and penicillamine structures.
- The side chain of penicillins plays a large part in the specificity of immunological reactions to the drugs. Allergic recognition of side chain determinants highlights the importance of including different individual penicillins in the battery of penicillin skin test reagents.
- IgE antibodies in the sera of patients allergic to β-lactam antibiotics detect a spectrum of antigenic specificities and IgE in the sera of different allergic patients shows heterogeneous recognition and cross-reactive responses. Some antibodies recognize discrete regions of the penicillin molecule such as the side chain only, or the thiazolidine ring only, while others show combining sites complementary to compound structures made up of the side chain with the β-lactam ring, the combination of the β-lactam and thiazolidine rings, or the whole molecule.
- IgE may also distinguish fine structural features on different penicillins, for example, IgE antibodies in the sera of allergic patients that distinguished amoxicilloyl and amoxicillanyl determinants.
- Patients with a history of prior reactions to penicillins have a four- to sixfold increased risk of a reaction to a penicillin compared to those without a previous history of hypersensitivity to the drugs.

- In September 2009, full regulatory approval was granted for penicilloyl-polylysine (Pre-Pen®) by the FDA. In Europe, a new commercial kit containing both the major determinant (5×10^{-5} M) and minor determinant mix (each component at a maximum concentration of 2×10^{-2} M) is now available. In September 2011, Pre-Pen® was approved by Health Canada. In 2011, an agreement with global distribution rights was reached for marketing the major determinant together with a minor determinant mix currently under development in the USA.
- For those with a negative history of penicillin allergy, the incidence of positive skin tests is 2–7 %. For skin test-positive patients given a therapeutic dose of penicillin, the risk of an acute allergic reaction ranges from 10 % in patients with a negative history to 50–70 % in patients with a positive history.
- The commercially available Phadia ImmunoCAP® drug-solid phases detect IgE antibodies to penicilloyl G and V, amoxicilloyl, and ampicilloyl determinants. These assays measure specific IgE antibodies in the range 0.01–100 kUA/l with a cutoff value of 0.35 kUA/l for a positive result. Levels higher than 0.1 kUA/l, indicate sensitization to the drug.
- When applied to the diagnosis of immediate hypersensitivity to β-lactams, the basophil activation test demonstrated a sensitivity of about 50 % and specificity of ~90–100 %. A high incidence of false positives has been observed.
- Challenge (provocation) testing should be performed only after prior skin testing and preferably a drug-specific IgE antibody test. If either of these tests returns a positive result that is in accordance with the patient's history, the risk precludes provocation testing. In the first instance, skin and IgE testing should be undertaken with benzylpenicillin and, if this is positive, the patient should be considered to be allergic to the β-lactam group of drugs. If testing with the parent penicillin is negative, the patient is then tested with the drug that provoked the reaction if it is known.
- The starting dose for penicillin desensitization is commonly about one ten-thousandth or less of a full therapeutic dose. Starting with a dose of 0.05 mg, doubling doses are given at 15 min intervals in 14 steps up to a maximum dose of 400 mg and a cumulative dose of 800 mg before administering the full therapeutic dose of the drug 30 min after the last desensitizing dose.
- Delayed-type, non-IgE-mediated hypersensitivity, often manifesting as macular or maculopapular exanthemata, may occur during treatment with penicillins, particularly aminopenicillins. Other delayed reactions include acute generalized exanthematous pustulosis, delayed urticaria/angioedema, exfoliative dermatitis, and the more severe bullous exanthems, Stevens–Johnson syndrome, and toxic epidermal necrolysis. Severe systemic hypersensitivity responses include vasculitis, hepatitis, interstitial nephritis, and pneumonitis. Penicillin-induced drug reaction (rash) with eosinophilia and systemic symptoms (DRESS) also occurs.
- Intradermal tests with delayed reading, patch tests, and occasionally prick tests provide the mainstay diagnostic procedures for the evaluation of delayed reactions to penicillins and other β-lactam drugs.
- Investigations on benzylpenicillin-specific T cell clones showed that processing of the free drug was not required whereas benzylpenicilloyl–HSA conjugate must undergo processing to stimulate T cell clones specific for this determinant.
- The HLA-B*57:01 genotype is a major determinant of drug-induced liver injury due to flucloxacillin.
- There is a significantly higher risk of a reaction to a cephalosporin in patients already allergic to penicillin. Second- and third-generation cephalosporins appear to carry less risk of provoking a reaction.
- Aminolysis of cephalosporins in the presence of polylysine or protein produces unstable intermediates that decompose to penaldate and ultimately penamaldate structures. This results in structures in which only the R1 side

chain, the attached amide, and remnants of the β-lactam ring remain from the original cephalosporin molecule. Immunochemical studies revealed clear IgE antibody recognition of cephalosporin R1 side chains and cross-recognition of β-lactam R1 groups showing structural similarity. The allergenic importance of cephalosporin R1 side chains was further supported by IgE recognition of synthetic hapten structures.

- With some cephalosporin-allergic patients, in addition to recognition of the R1 structure, the R2 side chain and/or the whole cephalosporin molecule are recognized by serum IgE antibodies. This was demonstrated by employing drug-solid phases prepared by linking HSA to the carboxyl group at position 4 on the cephalosporin nucleus.
- A cephalosporin concentration of 2 mg/ml for both prick and intradermal testing seems to be satisfactory in terms of sensitivity and specificity. Less than 20 % of patients with immediate reactions to cephalosporins react to benzylpenicillin, amoxicillin, and ampicillin, suggesting that testing with penicillins does not reliably predict cephalosporin allergy unless the side chains of the penicillin and the culprit cephalosporin are similar.
- Cephalosporins can cause delayed hypersensitivity reactions manifesting mainly as maculopapular or morbilliform rashes and delayed urticaria/angioedema. Intradermal tests are useful for identifying the cephalosporin responsible for delayed reactions. Patch tests are not recommended for cephalosporin-induced maculopapular and urticarial rashes.
- The monobactam aztreonam shows little, if any, cross-reaction with penicillins and cephalosporins, indicating that the β-lactam nucleus is not recognized. The side chain of the drug, not the core, is the main immunogenic site.
- Caution should be exercised in administering aztreonam to cystic fibrosis patients allergic to other β-lactam antibiotics because it is potentially allergenic on repeated use.
- The two most used carbapenems are the broad spectrum antibiotics imipenem and meropenem.
- The 0.9 % incidence of positive reactions found for imipenem is significantly lower than previous estimates and lower than the 4.4 % rate of cross-reactivity claimed for penicillins and cephalosporins. Skin testing and challenges showed that reactivity to meropenem and penicillins in patients with IgE-mediated allergy to penicillins was no higher than 5.2 % and the data indicated that no more than 3.5 % of patients would have a positive challenge after a negative skin test. On the basis of these findings, it has been suggested that the practice of avoiding imipenem and meropenem therapies in penicillin-allergic patients should be reconsidered.
- It seems likely that IgE antibodies to imipenem and meropenem generally recognize the groups attached at C-6 and to the sulfur atom at C-3 of the bicyclic nuclear structure.
- There appears to have been an increase in cases of immediate hypersensitivity to clavulanic acid, leading to speculation that this is a reflection of its increase in usage with amoxicillin.
- There are a small number of reports of delayed reactions to clavulanic acid manifesting as generalized urticaria, generalized itchy erythema, and allergic contact dermatitis with pruritic erythematous patches.
- The combination of amoxicillin and clavulanic acid is associated with the risk of drug-induced liver injury, mostly cholestatic in nature.

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Other Antimicrobial Drugs

6

Abstract

Allergic reactions to commonly used antibiotics such as the tetracyclines, macrolides, and rifamycins are rare. Neomycin consistently ranks in the top 10 % of the most common causes of allergic contact dermatitis while the incidence of bacitracin allergy is in the range 8–9.5 %. The most commonly occurring adverse effects caused by vancomycin are termed, collectively, red man syndrome. Immediate type I allergy is the most well-defined sulfonamide-induced hypersensitivity reaction with the best defined allergenic drug structures. Antigen-presenting cells may produce sulfonamide hapten–protein antigen complexes and ultimately induce the T cell response to the drug. Cross-reactions with a wide variety of frequently used non-antibacterial drugs containing a common sulfonamide group are thought not to occur. Almost all reports of trimethoprim-induced hypersensitivities are of the immediate kind and three different trimethoprim allergenic determinants structures have been identified. IgE antibodies apparently specific for quinolones and positive skin tests in apparently normal, healthy controls have been demonstrated. Immediate type I mechanisms are responsible for the most important chlorhexidine-induced allergic reactions. The whole molecule has been identified as the allergenic determinant. In almost all cases of povidone–iodine hypersensitivity, polyvinylpyrrolidone not iodine has been implicated as the offending component.

For both the clinician and patient, an allergic reaction elicited by a β-lactam antibiotic is probably considered the archetypal drug allergy but a range of other antibiotics, both naturally occurring and semisynthetic, and a number of other antimicrobials of varied origin and nature also provoke a spectrum of immediate and delayed allergic responses. Although collectively this group of drugs distinct from the β-lactams is large and chemically diverse, an attempt is made in this chapter to consider them under the broad heading of other antimicrobials.

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_6, © Springer Science+Business Media, LLC 2013

6.1 Antibiotics

The definition we will use here for an antibiotic (see also Chap. 5) is a chemically defined substance produced by, or derived from, certain bacteria, fungi, or other organisms (not necessarily microorganisms), or produced semi-synthetically, that can, in low concentrations, destroy or inhibit the growth of other microorganisms. This definition excludes chemotherapeutic antibacterials such as the sulfonamides that appear to be increasingly referred to as antibiotics. Antibiotics seem to have always been around and although our immediate thoughts about them are positive and we naturally think of them as one of the mainstays of chemotherapy for infections of man and animals of all sorts, the nagging warnings of their overuse and consequent loss of effectiveness against some major life-threatening bacteria are a sobering reminder that these agents can never be taken for granted. In fact, antibiotics have been used for about 70 years during which time they have revolutionized the treatment of infectious diseases, but, in addition to loss of effectiveness that results from their selection of resistant organisms, like many drug, they have provoked a catalog of adverse reactions from transient rashes to life-threatening anaphylaxis.

In terms of usage, no other antibacterial agent rivals the prolonged and heavy prescribing of penicillins and cephalosporins so it might be expected that adverse reactions to non-β-lactam antibacterials would be encountered less often and this appears to be the case. The word "appears" has been used deliberately here; comparing, for example, the number of reports of allergic reactions to clindamycin and amoxicillin draws attention to the need for a standardized method of comparison and this ultimately depends on the number of reactions for a specified number of administrations. Calculation and comparisons of such frequencies of reactions are not often done, but, even so, the sparse reports of allergic reactions to commonly used antibiotics such as the tetracyclines and macrolides and frequently used antimicrobials such as chlorhexidine do indeed suggest that the reactions are rare. There have been times when some non-β-lactam antibiotics were described as being implicated in hypersensitivity reactions with moderate frequency. This was true for both streptomycin and gentamycin, but their decreasing usage has seen reactions to these drugs decline significantly. The same decline related to usage has been seen with kanamycin, lincomycin, and polymyxins which were implicated in reactions occasionally but which are now rarely, if ever, a problem. By contrast, bacitracin, once thought of as an offender of low incidence, has now become recognized as the largest cause of hypersensitivity reactions induced by drugs used topically. With this big increase in cases there has also been an alarming number of reports of anaphylaxis, something that could surely not have been anticipated. In relation to anaphylaxis, recently published large epidemiologic surveys from France of drugs causing anaphylaxis during anesthesia showed that despite the number of reactions to neuromuscular blocking drugs and latex (the agents responsible for the large majority of cases) remaining relatively stable, the incidence of anaphylaxis to antibiotics has increased significantly during the same time period.

6.1.1 Macrolides

Erythromycin, from the actinomycete *Saccharopolyspora* (formerly *Streptomyces*) *erythraea*, is the first member of this family of antibiotics to be marketed and successfully used clinically to treat infections in humans. It has an antimicrobial spectrum at least as wide as the penicillins, and interestingly, from our perspective, it is often used as a replacement for patients allergic to that group of drugs. Besides erythromycin, other members of the macrolide family of antibiotics that are clinically useful include azithromycin, clarithromycin, dirithromycin, roxithromycin, telithromycin (these six are approved by the FDA), oleandomycin, and spiramycin. Clarithromycin, dirithromycin, and roxithromycin and the azalide azithromycin are more recent members of the group and can be regarded as newer generation macrolide antibiotics.

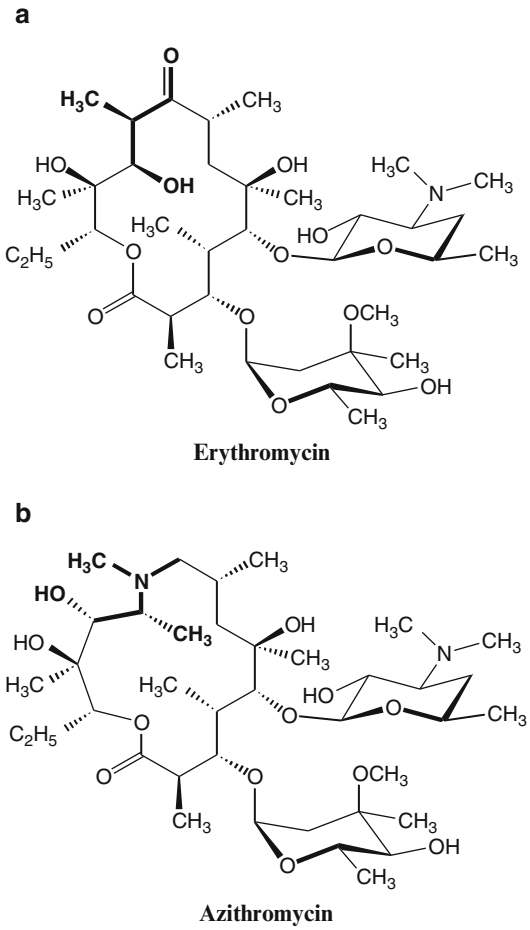


Fig. 6.1 Structure of the macrolide antibiotic erythromycin (**a**) which contains a 14-membered lactone ring. The azalide, azithromycin (**b**), derived from erythromycin, has an *N*-methyl group incorporated into the lactone ring, making it a 15-membered ring (compare *highlighted* sections)

6.1.1.1 Structures

Chemically, the macrolide antibiotics comprise a large cyclic lactone ring structure with one or more molecules of the unusual sugars, L-cladinose and D-desosamine, attached by glycosidic linkage. The lactone ring structure may be from 12- to 16-membered—clarithromycin, dirithromycin, erythromycin, oleandomycin, roxithromycin, and telithromycin each have a 14-membered lactone ring while azithromycin is a 15-membered and spiramycin a 16-membered macrolide. Figure 6.1a shows the structure of the 14-membered lactone erythromycin. Changes to its structure, sometimes small, produce other useful members of the family. The structure of azithromycin, now one of the most heavily used antibiotics, has a nitrogen atom in the 15-membered macrolide ring making it an azalide (Fig. 6.1b).

6.1.1.2 Macrolide Hypersensitivities

Allergies to macrolide antibiotics are said to occur with an incidence of 0.4–3 %. Apart from the very occasional case report of anaphylaxis, the most commonly seen symptoms of a hypersensitivity reaction to this group of drugs include urticaria, often generalized, angioedema, pruritus, asthma, tachycardia, and delayed cutaneous reactions presenting as maculopapular exanthema. Stevens–Johnson syndrome and toxic epidermal necrolysis have been observed rarely. Drug-reactive serum IgE antibodies to erythromycin inhibited by the drug have been demonstrated in at least one case and the Prausnitz–Kustner test was shown to be positive in a case of anaphylaxis following ingestion of a single dose of the drug. The presence of macrolide-reactive IgE antibodies in some patients has also been inferred from positive prick test results, in particular, in studies involving spiramycin and roxithromycin. Skin prick tests were also utilized to examine cross-reactivities of spiramycin and roxithromycin each with clarithromycin and erythromycin. Cross-reactivity was absent in the former case but present in the latter. A number of cases of asthma to spiramycin, generally in an occupational setting in pharmaceutical plants, have been reported and confirmed by one or more of skin tests and/or challenge (nasal or inhalation) tests. An anaphylactoid reaction to telithromycin, a semisynthetic derivative of erythromycin and the first ketolide antibiotic to be used clinically, was notable because of the symptoms of shortness of breath, wheezing, and angioedema that occurred after the first dose even though the patient had taken erythromycin and azithromycin in the past without adverse effects.

6.1.1.3 Diagnosis of Macrolide Hypersensitivities

The foregoing brief summary indicates the gaps in our knowledge of macrolide hypersensitivities, gaps brought about not only by the paucity of patients to study but also by the lack of studies designed to investigate the underlying mechanisms, our ignorance of the allergenic structural determinants and the absence of good clinical follow-up. For the macrolides, skin tests, prick, intradermal, and patch, are said to yield positive results in only 25–50 % of patients and this brings into focus the question of which examinations *are* of value in confirming a diagnosis of macrolide allergy. Skin testing in cases of suspected allergies to erythromycins is poorly standardized as acknowledged in a recent study recommending 0.05 mg/ml as a suitable nonirritating concentration of clarithromycin for intradermal testing. Erythromycin-reactive IgE antibodies specifically inhibited by the antibiotic and cross-reactive with the structurally related diacetylmidecamycin were demonstrated with a solid phase immunoassay prepared by reacting erythromycin lactobionate with bis-oxirane-activated Sepharose.

Recent studies designed to test what was seen as the possible uncritical assignation of macrolide hypersensitivity concluded that the diagnosis was often misinterpreted and lacked the necessary confirmatory data. Essentially, a diagnosis was often said to be made without diagnostic evaluation and based on nothing more than a suggestive history of a temporal relationship between macrolide intake and symptoms. For the moment at least, the conclusion seems to be that history alone has been too often used in the diagnosis of macrolide hypersensitivity and the number of patients so classified is a healthy overestimate. In addition, skin and in vitro tests such as serum IgE antibody determination, the basophil activation test, lymphocyte transformation tests, and the tryptase assay were judged to be not very useful in identifying hypersensitive patients. The challenge test alone, regarded as "the gold standard," is advocated as necessary to definitely confirm or rule out allergy to macrolides. The design and logic of the thinking and the studies behind this reexamination of the approach to the diagnosis of macrolide hypersensitivity are hard to disagree with and yet the surprisingly small numbers of patients with true macrolide allergy distinguished by provocation tests and excluded by skin and laboratory tests might be as much a reflection of the starting cohort of patients as an indication of the inappropriateness of applied tests. Starting with patients referred on by general practitioners and other clinicians, many of whom are not allergists, almost certainly means that the initial applied diagnostic criteria will be varied, perhaps highly so, and perhaps based on no more than the temporal relationship already referred to. It seems likely that the allergological assessments undertaken by the general practitioners will be less thorough and appropriate than those undertaken by the allergy specialists. Under these circumstances it seems hasty to conclude that skin and in vitro tests are not helpful in establishing a diagnosis. This would appear to be true for all allergens if the patients were poorly or inappropriately assigned in the first place.

6.1.2 Tetracyclines

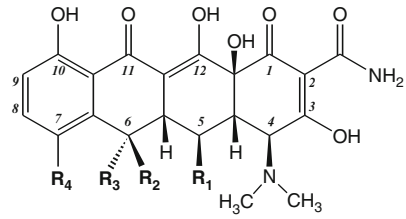
6.1.2.1 Adverse Reactions

Even though minocycline has been identified with a number of serious adverse drug reactions including hypersensitivity and there are a small number of reports of anaphylaxis to tetracyclines, this family of broad spectrum antibiotics, developed in the 1940s, is seen as being comparatively safe, especially when viewed against their long-term and frequent usage. Dermatologists first prescribed tetracyclines in the early 1950s, when it became apparent that the drugs offered an effective treatment for acne vulgaris. Minocycline, now widely used for this purpose, has been implicated in a serum sickness-like reaction, drug-induced lupus, cases of single organ dysfunction, and the so-called drug hypersensitivity syndrome reaction, a severe adverse drug reaction that may occur following a number of different medications including anti-epileptic drugs, sulfonamides, dapsone, azathioprine, allopurinol, and cyclosporin. A variety of clinical abnormalities

may be seen in drug hypersensitivity syndrome, in particular, fever, skin lesions, lymphadenopathy, and internal organ involvement. Skin reactions reported include exudative maculopapules, purpuratous macules, and erythema multiforme-like plaques and other reported effects on organs include eosinophilia, agranulocytosis, atypical lymphocytosis, autoimmune hyperthyroidism, hepatitis, nephritis, and myositis. In important studies from Canada, 19 cases of hypersensitivity reaction were assessed to be due to minocycline, two to tetracycline, and one to doxycycline. Of 16 cases of serum sickness-like reaction, 11 were due to minocycline, 3 to tetracycline, and 2 to doxycycline. For single organ dysfunction, 40 were caused by minocycline, 37 by tetracycline, and 6 by doxycycline, and all 33 cases of drug-induced lupus were found to be due to minocycline. Despite the infrequency of these reactions, the large numbers of subjects, especially teenagers, taking minocycline is a reminder for the ongoing need to remain aware of possible adverse reactions with this drug.

6.1.2.2 Structural Considerations

It has been suggested that these serious adverse effects of minocycline might be due to a reactive metabolite(s), but this concept remains vaguely defined and so far unsupported by experimental findings. When the structures of the clinically used members of the family are examined (Fig. 6.2), one feature in particular, the 4-dimethylamino group present in all four compounds shown, stands out as a likely immunologically reactive substituent. IgE antibody recognition of tertiary and quaternary substituted ammonium groups is well known (Sects. 7.4.2 and 8.5) and reaction of tetracycline and doxycycline with IgE in the sera of subjects with multiple allergic drug recognition profiles has been demonstrated. Drug interaction with IgE was shown to be due to antibody combining site recognition of tertiary and quaternary mono-, di-, and trialkyl groups, but only if the alkyl groups were "small," namely, methyl or perhaps ethyl. Using such haptens immobilized on a solid phase, IgE antibodies have also been detected in sera from subjects with a suspected allergy to



	-R_1	-R_2	-R_3	-R_4
Tetracycline	-H	-CH_3	-OH	-H
Oxytetracycline	-OH	-CH_3	-OH	-H
Doxycycline	-OH	-CH_3	-H	-H
Demeclocycline	-H	-H	-OH	-Cl
Minocycline	-H	-H	-H	-$N(CH_3)_2$

Fig. 6.2 Structures of tetracyclines used clinically. Note that all four compounds contain a 4-dimethylamino group but minocycline has a second one, raising the possibility of allergenic bivalency

doxycycline. In addition, with minocycline, the presence of a second dimethylamino group at position 7 (position R_4 in Fig. 6.2) raises the possibility of allergenic bivalency, that is, the presence of two potentially reactive determinants that might not only bind to their complementary IgE antibody combining sites but also cross-link the antibodies on the surfaces of mast cells and basophils. In other words, the free drug might elicit the direct release of inflammatory mediators without the presumed necessary binding to a carrier to form an antigenic complex. The subject of immunological recognition of allergenically bivalent drugs is discussed in greater detail in Sect. 7.4.2.3.

6.1.3 Rifamycins

Rifamycins belong to a group of antibiotics some of which are biosynthesized in fermentation cultures by the bacterium *Amycolatopsis rifamycinica* while some are semisynthetic compounds prepared by derivitization. The name of the bacterium that produces rifamycins has evolved from the original *Streptomyces mediterranei* to *Nocardia mediterranei*, then to *Amycolatopsis mediterranei*, and in 2004 to *A. rifamycinica*.

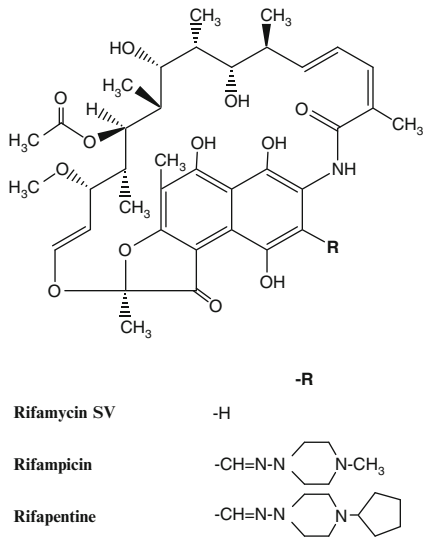


Fig. 6.3 Structures of rifamycin SV, a rifamycin B structure, and starting point for rifampicin which has a 4-methyl-1-piperazinaminyl side chain. The semisynthetic rifapentine is also used clinically

Fermentation produces five chemically different but structurally closely similar rifamycins designated A, B, C, D and E. Rifamycin SV is a derivative of the B structure and is the starting point for the formation of rifampicin that has an added 4-methyl-1-piperazineaminyl side chain. The chemical structures of rifamycin SV and rifampicin are shown in Fig. 6.3. Three other semisynthetic rifamycins, rifabutin, rifapentine, and rifaximin are also used clinically. The rifamycins are broad spectrum antibiotics used to treat tuberculosis, gonorrhea, leprosy, and respiratory and biliary tract infections. They are active against *Helicobacter pylori* and used for anti-infective prophylaxis against meningococcal infection. Other important dosage forms are as eye drops for the treatment of infectious conjunctivitis and as local applications to treat infected surgical and traumatic wounds.

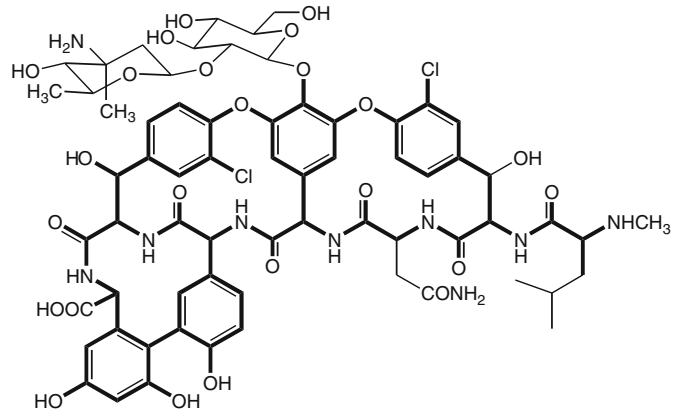
Adverse reactions to rifamycins suggested to be immune mediated include a "flu"-like syndrome, acute renal failure, hemolytic anemia, and thrombocytopenia. Other more typical manifestations of hypersensitivity include urticaria, contact dermatitis, erythema multiforme, vasculitis, and rarely Stevens–Johnson syndrome and toxic epidermal necrolysis. The FDA has warned that rifampicin should not be given intermittently and the daily dosage regimen should not be interrupted since renal hypersensitivity reactions may occur when therapy is resumed. Rifamycin SV and rifampicin have provoked a number of cases of anaphylaxis, usually following local administration, and the presence of IgE antibodies in some patients has been indicated by one or more of a positive skin prick, Prausnitz–Kustner test, basophil activation, and/or a radioallergosorbent test. For rifampicin, an irritant-free intradermal test concentration of 0.002 mg/ml has been recommended. In one procedure used for a rifamycin radioallergosorbent test, rifamycin SV was conjugated to poly-L-lysine before employment of reductive amination to reduce the Schiff base. The drug–carrier conjugate was then covalently coupled to activated cellulose and used in an immunoassay to detect serum IgE antibodies in patients' sera. Immunochemical identification of IgE antibody-binding fine structural determinants has not yet been attempted, but investigations over 35 years ago in Italy identified rifamycin SV-reactive IgE antibodies in the sera of patients allergic to rifampicin. The antibodies recognized the chromophoric nucleus of the rifamycin molecules but not the 4-methylpiperazine side chain (Fig. 6.3). The only other experimental findings relevant to the allergenic structures of rifamycins were the recent demonstration of cross-reacting IgE antibodies in the serum of a patient who experienced an anaphylactic reaction to rifamycin SV solution applied to a wound and an immediate response involving urticaria and dyspnea following the ingestion of a tablet of rifaximin. Rifamycin SV and rifampicin reacted directly with the serum IgE antibodies while rifabutin, rifapentine, and rifaximin all demonstrated significant inhibition of antibody binding to a rifampicin–Sepharose solid phase.

6.1.4 Vancomycin and Teicoplanin

6.1.4.1 Structures

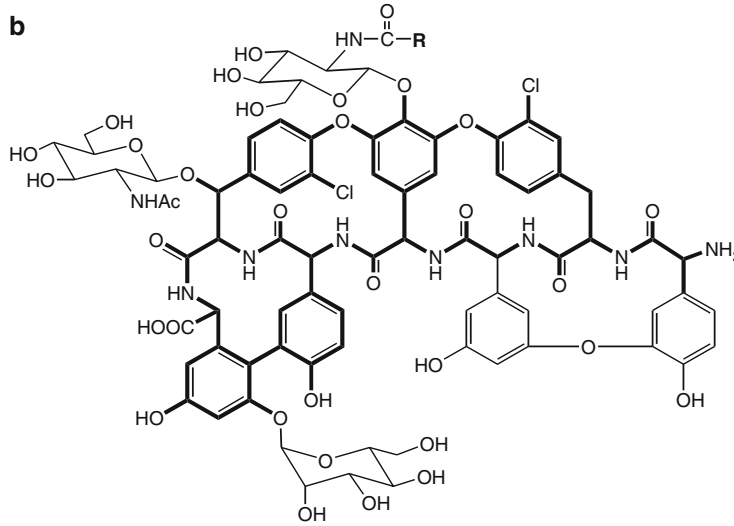
Vancomycin, a tricyclic glycopeptide antibiotic (Fig. 6.4a) produced by *Amycolatopsis* (formerly

a



Vancomycin

b



Teicoplanin

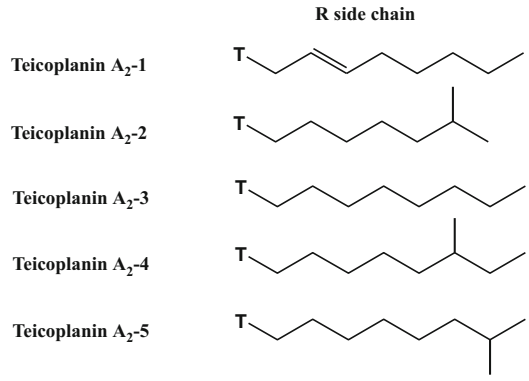


Fig. 6.4 Vancomycin (**a**) is a tricyclic glycopeptide antibiotic. The aglycone consists of a heptapeptide connected by six peptide bonds with several non-proteinogenic amino acids, five of which are aromatic. D-Glucose and the novel amino sugar vancosamine are linked to the aglycone through glycosidic bonds. Teicoplanin (**b**), a lipoglycopeptide, is a mixture of several compounds sharing the same glycopeptide core. Three sugar moieties are present, *N*-fattyacyl-β-D-glucosamine, *N*-acetyl-β-D-glucosamine, and D-mannose. The common core structure shared by the two antibiotics is highlighted in *bold*

Streptomyces and then *Nocardia*) *orientalis*, is often thought of as the "drug of last resort" for the treatment of pathogens such as methicillin-resistant *Staphylococcus aureus*. The aglycone part of the vancomycin structure consists of a heptapeptide connected by six peptide bonds with several non-proteinogenic amino acids, five of which are aromatic. The sugars present, D-glucose and the novel amino sugar vancosamine, are linked to the aglycone through glycosidic bonds and are thought to contribute to ligand binding and a consequent enhancement of antimicrobial activity. Teicoplanin, a lipoglycopeptide, is a mixture of several compounds sharing the same glycopeptide core. Three sugar moieties are present, *N*-fattyacyl-β-D-glucosamine, *N*-acetyl-β-D-glucosamine, and D-mannose (Fig. 6.4b).

6.1.4.2 Red Man Syndrome

The most commonly occurring and well-known adverse effects caused by vancomycin are referred to collectively as red man (or neck) syndrome. In terms of severity, reactions may range from mild pruritus, erythema, and flushing of the upper body (Fig. 6.5) to angioedema and rarely hypotension and cardiovascular collapse. Patients often complain of diffuse itching with a burning feeling and experience headache, dizziness, chills, fever, and general discomfort. Signs and symptoms usually occur 5–10 min after the commencement of infusion of the drug or they may become apparent after infusion is complete. The visual signs of the vancomycin-induced reactions, the known pharmacological effects of histamine, demonstrations that the antibiotic causes degranulation of rat peritoneal mast cells, and the absence of drug-reactive IgE antibodies all indicate that red man syndrome is caused by the release of histamine as a result of degranulation of mast cells and basophils. A further indication that the reactions are anaphylactoid rather than immune mediated, that is, anaphylactic, is the demonstration that tryptase levels are generally not increased during vancomycin-induced anaphylactoid reactions although the drug induces tryptase release from mast cells in vitro. Note, however, that this finding does not correlate with in vivo studies. (For more on the relationship between tryptase and anaphylactoid reactions see Sect. 4.5.1 and Sect. 7.4.3.2). The incidence of reactions has been estimated to be from 3.7 to 47 % of patients with the most severe reactions occurring in those less than 40 years old, particularly in children. Oral administration of vancomycin led to red man syndrome in a neutropenic child with normal renal function and it has been claimed that between 50 and 90 % of normal and infected adults might have a reaction to the drug. Following administration of 1 g of vancomycin over 1 h to healthy volunteers, 80–90 % showed signs and symptoms of red man syndrome. Patients being treated for infections appear to have a lower and less severe reaction rate.

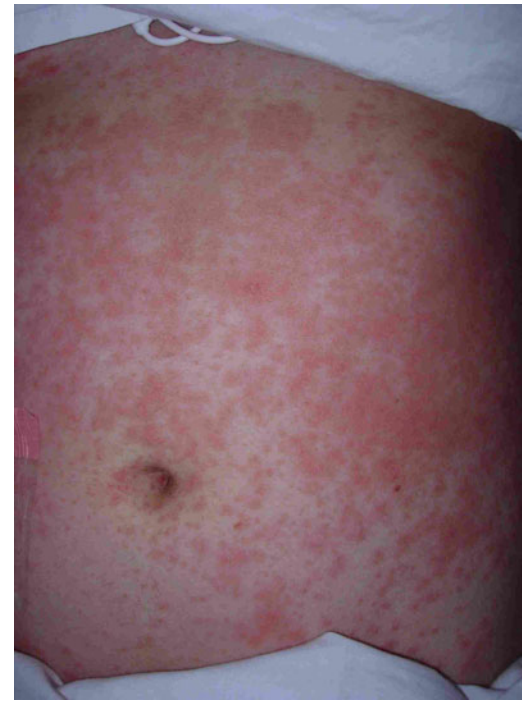


Fig. 6.5 Red man syndrome on the upper part of the body following vancomycin administration (photograph courtesy of Dr. F. C. K. Thien and Department of Dermatology, Alfred Hospital, Melbourne)

It became apparent that appearance of reactions, and their severity, were subject to both the dose of vancomycin and the rate of its infusion. A clinical study involving the measurement of plasma histamine levels every 10 min during the first infusion of each regimen revealed that the largest increases in histamine levels occurred in subjects given 1 g doses; those given half that

amount showed only slight changes in histamine levels. A significant relationship was also seen between histamine release and reaction severity. Findings such as these have led to the recommendation that vancomycin should not be administered as a bolus and quantities such as 1 g should be infused over a 1-h period. Some other drugs such as rifampicin, ciprofloxacin, amphotericin B, and rarely teicoplanin (see also below) may cause red man syndrome and these drugs, and others such as neuromuscular blockers, opioid analgesics, and contrast media, can accentuate reactions by provoking the release of histamine (see Sects. 3.2.5.1, 7.3, 8.4 and 10.4.1.1). Reactions may be prevented or their severity decreased by extending the infusion time and/or premedication with histamine antagonists such as the H_1 receptor blocker diphenhydramine alone or combined with the H_2 receptor antagonist cimetidine. Despite its chemical similarity with vancomycin, teicoplanin is claimed not to cause red man syndrome and histamine release even when infused at rates significantly faster than the rates employed for vancomycin and this has led to its recommendation as a substitute for patients intolerant to the latter drug. In a comparison of the two drugs, vancomycin, 15 mg/kg administered over 60 min, and teicoplanin, 15 mg/kg over 30 min, were compared in a double-blind, randomized, two-way crossover study to determine the occurrence and severity of red man syndrome and histamine release. Vancomycin caused red man syndrome in 11 of 12 patients and provoked significant release of histamine into the plasma. Teicoplanin did not cause either the syndrome or significant histamine release.

6.1.4.3 Other Adverse Reactions to Vancomycin and Teicoplanin

Despite a number of reports that cover a range of systemic and dermatologic reactions, severe reactions including true type I responses are rare. An anaphylactoid reaction to infused vancomycin reported in a patient with vancomycin-induced red man syndrome was interpreted, somewhat obscurely, as a possible case of true vancomycin allergy. Other cases induced by vancomycin, but also showing cross-sensitization with teicoplanin, have been judged to be type I responses. A case of direct contact allergy involving periorbital skin erosive rash, hyperemia of conjunctiva, and corneal stromal edema, and manifesting as itch, soreness, burning, and photophobia, was induced by vancomycin eye drops. Symptoms resolved upon withdrawal of the eye drops and upon initiation of intravenous teicoplanin which was tolerated, perhaps surprisingly given its structural similarity to vancomycin. Skin tests with vancomycin were positive indicating a type I hypersensitivity but skin tests have not yet been validated with vancomycin, a known histamine releaser, so a number of questions related to concentration and irritation remain.

Other reports of adverse reactions to vancomycin include renal disorders, drug fever, and hematological disorders including eosinophilia, immune thrombocytopenia, leukocytosis, neutropenia, and drug hypersensitivity syndrome (also called drug rash with eosinophilia and systemic symptoms [DRESS] syndrome; Sect. 2.2.4.1), the latter being resolved in one study by substituting teicoplanin. The most common hypersensitivities to vancomycin are cutaneous reactions which may be a mild skin rash, the more frequent maculopapular or urticarial skin eruptions, exfoliative dermatitis, fixed drug eruption, vasculitis, and the rare reactions such as toxic epidermal necrolysis, Stevens–Johnson syndrome, and linear IgA bullous dermatosis which can be confused with the first of these toxic bullous cutaneous reactions.

The chemically similar teicoplanin, not approved in the USA, is not inferior to vancomycin with regard to efficiency of treating gram-positive infections. It shows a lower rate of adverse reactions, particularly nephrotoxicity and, as already discussed, is used as a substitute for vancomycin in red man syndrome. When hypersensitivity reactions do occur with teicoplanin they are generally of the delayed type, but there are a few documented cases of apparent IgE antibody-mediated reactions implicated, for example, by an immediate wheal and flare skin reaction to the drug or by teicoplanin-induced histamine release from a patient's basophils. Despite the chemical and pharmacological

similarities of the drugs, a reaction to teicoplanin with tolerance to vancomycin has been documented. The reaction occurred 2 days after administration of teicoplanin and presented as a maculopapular exanthema on the trunk and arms over a 7-day period. Skin prick, intradermal, and patch tests with the drug were negative, but an intravenous challenge test with 400 mg of teicoplanin elicited pruritic erythematous papular exanthemata on the elbows, forearms, and abdomen 1 h after administration. A later control challenge with vancomycin up to the therapeutic dose demonstrated good tolerance to the drug. This case was unusual since the literature reveals that the two drugs cross-react almost invariably in conditions such as maculopapular exanthemata, vasculitis, acute generalized exanthematous pustulosis, and drug hypersensitivity syndrome.

6.1.4.4 Skin Tests with Vancomycin and Teicoplanin

Skin testing with vancomycin and teicoplanin has not been well studied and the procedure remains to be validated with both positive and negative predictive values unknown. Skin test results, and particularly details of drug concentrations used and methodologies employed, are hard to find in the vancomycin–teicoplanin literature on adverse reactions. In a case study of vancomycin anaphylaxis followed by successful desensitization, intradermal skin tests with the drug were positive at a concentration of 0.1 μg/ml. Control subjects showed positive responses at concentrations of 10 μg/ml or greater. A loss of skin test reactivity to vancomycin has been demonstrated in one case study after successful desensitization to the drug.

6.1.4.5 Desensitization for Vancomycin Hypersensitivity

There are occasions with a drug like vancomycin when desensitization is appropriate or even required. Circumstances where desensitization would be considered include anaphylaxis to vancomycin and the difficult situation where a case of red man syndrome cannot be overcome by slowing the infusion rate of vancomycin, where premedication with histamine antagonists proves poorly effective or ineffective and when no other available antimicrobial agent is effective against the infective organism. Desensitization is usually used in cases of immunoglobulin E-mediated reactions to antibiotics, and although it can effectively induce tolerance to the problematic drug, the mechanism by which this occurs with drugs such as vancomycin is still to be understood and explained (see Sect. 3.5). Both rapid (carried out over several hours) and slow (over a period of days) desensitization protocols have proved effective, although the former is preferred since it enables therapy for acutely ill patients to continue within 24 h. Contraindications to be aware of before the initiation of desensitization to vancomycin include a history of leukocytoclastic vasculitis, extensive fixed drug eruption, drug-induced hypersensitivity syndrome, and past exfoliative skin reaction such as Stevens–Johnson syndrome and toxic epidermal necrolysis. Desensitization should be undertaken immediately before the desired therapy. Other important considerations are the patient's health issues, particularly any preexisting cardiac and pulmonary conditions, the patient should not be taking any drugs that might increase the chance of anaphylaxis or interfere with its treatment (for example, beta-blockers and ACE-inhibitors) and desensitization should be carried out with informed consent in an appropriate setting such as an intensive care unit with all necessary resuscitation equipment and drugs on hand. Concomitant administration of histamine releasing drugs such as neuromuscular blockers, opioid analgesics, some plasma expanders, propofol, contrast media, and antibiotics such as ciprofloxacin should be avoided or the drugs discontinued or given in smaller doses.

6.1.4.5.1 Protocols for Desensitization

In one early successful procedure that can be completed in approximately 4 h if each dose is tolerated and no repeats are needed, the patient is premedicated with diphenhydramine 50 mg iv and hydrocortisone 100 mg iv 15 min prior to starting and 6 hourly thereafter. Starting with a 250 ml solution of vancomycin 2 mg/ml, four successive tenfold dilutions are made up to

Table 6.1 Rapid desensitization protocol for vancomycin

Dose number	Dose (mg)[a]	Dose number	Dose (mg)[a]
1	0.005	11	5.00
2	0.010	12	10.00
3	0.020	13	20.00
4	0.040	14	40.00
5	0.080	15	80.00
6	0.160	16	160.00
7	0.320	17	320.00
8	0.640	18	640.00
9	1.25	19	1,000.00
10	2.50	–	–

[a]Each dose, except the final dose, was given in 50 ml of 5 % dextrose infused by infusion pump over 15 min. The last dose was 25 ml of a solution of 1 g vancomycin in 250 ml of 5 % dextrose. This was infused over 15 min and the remaining solution was then infused at a rate of 200 ml/h. Reproduced with permission from Villavicencio AT et al. J Allergy Clin Immunol 1997;100:853

produce solutions of vancomycin containing concentrations of 0.2 mg/ml, 0.02 mg/ml, 0.002 mg/ml, and 0.0002 mg/ml. That is, five different solutions are prepared ranging in concentration from 2 to 0.0002 mg/ml. Beginning with the most dilute solution, the drug is infused at a rate of 0.5 ml/min and this is increased by 0.5 ml/min every 5 min as long as the dose is tolerated. If an increased rate is not tolerated, the concentration is stepped down to the previously highest tolerated rate. This step is repeated up to three times for any given concentration. Upon completion of infusion of the solution of highest concentration (2 mg/ml), the patient's required dose of vancomycin is administered over 2 h with diphenhydramine 50 mg given orally 1 h before each dose. This procedure devised over 25 years ago (Lerner and Dwyer 1984) may be compared with a more recent rapid protocol summarized in Table 6.1 and successfully applied in a patient who experienced acute cardiac and pulmonary arrest after infusion of vancomycin. It should be recognized that mild and transient hypersensitivity reactions such as rash, pruritus, flushing, and erythema occur in about 30 % of patients during desensitization procedures, but if these symptoms are tolerated by the patient and do not cause too much discomfort, desensitization can continue. This is what happened using the desensitization protocol set out in Table 6.1 where the patient experienced itching that responded to intravenous diphenhydramine and mild hypotension and oxygen desaturation that required reversal with subcutaneous epinephrine and intravenous diphenhydramine. In comparison to the Lerner and Dwyer procedure, this protocol requires the preparation of many more dilutions of vancomycin. Not all patients tolerate a rapid desensitization and the proportion that do not is not known. In such patients, desensitization may still be achieved by employing so-called slow protocols where infusions rates and buildup of dose are far slower, often extending over many days. In the event of intolerance to a rapid desensitization procedure, desensitization to vancomycin has ultimately been achieved in some cases by switching to a slow protocol. To maintain the desensitized state and avoid the possibility of having to repeat the entire desensitization procedure, care should be taken to maintain the administration of vancomycin.

Long-term vancomycin therapy may induce neutropenia. A recent report of severe neutopenia following a prolonged course of vancomycin that progressed to agranulocytosis after reexposure to the drug should be kept in mind and focus attention on the safety of rechallenging vancomycin patients with possible drug-induced neutropenia.

6.1.5 Antibiotics Used Topically with Emphasis on Neomycin and Bacitracin

6.1.5.1 Neomycin

Neomycin, produced by the bacterium *Streptomyces fradiae*, is an aminoglycoside antibiotic that shows good activity against gram-negative bacteria and some gram-positives. Neomycin is made up of neomycin B and neomycin C. Hydrolysis of neomysin B yields neamine made up of neosamine B and the aminocyclitol, 2-deoxystreptamine, and the disaccharide neobiosamine B composed of D-ribose and neosamine B. Hydrolysis of neomycin C produces neamine and neobiosamine C, a disaccharide

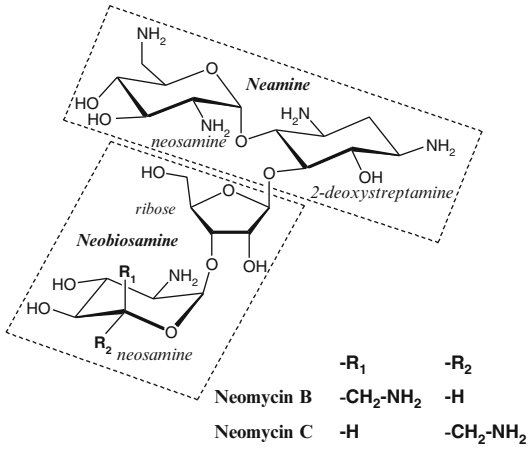


Fig. 6.6 Structure of the aminoglycoside antibiotic neomycin, made up of neomycin B and neomycin C. Each contains 2-deoxystreptamine while neomycin B is made up of neosamine B and neobiosamine B and neomycin C is composed of neosamine C and neobiosamine C. Neosamines B and C are stereoisomers relative to the amino group

composed of D-ribose and neosamine C. Neosamine B and neosamine C are stereoisomers relative to the amino group (Fig. 6.6). With the presence of a number of free amino and hydroxyl groups, the molecule readily lends itself to chemical manipulation for the preparation of antigens for diagnostic use.

Delayed Hypersensitivity Reactions to Neomycin

Allergic contact dermatitis, a type IV delayed hypersensitivity reaction, is an increasing problem arising from the application of agents during the care of postoperative wounds, both closed and open. Reports of neomycin as a causative agent date back to at least the 1960s. In fact, neomycin consistently ranks in the top 10 % of the most common allergenic causes of allergic contact dermatitis with patch test studies revealing sensitivities of 10–12 % in general patch test populations, in the postsurgical population, and in patients suspected of the condition. Allergic sensitization to neomycin in patients with chronic venous insufficiency has been reported to be as high as 34 % and it has been claimed that the drug ranks near the top with nickel as the most tested drug over the last 30 years. In addition to delayed eczematous contact dermatitis, neomycin is also known to cause generalized reactions such as exfoliative dermatitis and erythroderma. Patch tests on patients with chronic otorrhoea have shown positive reactions in up to nearly 60 % of subjects with neomycin and framycetin the major offenders. In a prospective study of delayed hypersensitivity reactions to topical aminoglycosides in patients undergoing middle ear surgery, 119 patients with chronic otitis media and 30 with otosclerosis were skin tested with a panel of aminoglycoside antibiotics. Overall, 14 % of patients were positive to at least one aminoglycoside with 13.4 % positive to gentamycin, 12.8 % to neomycin, and 4.5 % to gramicidin. Sixteen percent of patients with chronic otitis media were allergic to one of the aminoglycosides commonly found in antibiotic eardrops. Findings such as these have led to the suggestions that patch testing is almost obligatory in patients with long-standing otitis that does not respond to local therapy and, because of their high risk of sensitization, topical neomycin (particularly as eye drops—see Fig. 6.7) and framycetin should not be used routinely if at all. Attention has also been drawn to the need to keep patch tests in place for up to 7 days since the aminoglycosides need this longer time interval to reveal positive responders.

6.1.5.2 Bacitracin

The high levels of allergic sensitivity provoked by neomycin and gentamycin naturally led to efforts to find a less allergenic, but equally effective, substitute. Initially, the antibiotic bacitracin seemed to satisfy these requirements. Bacitracin is not an aminoglycoside but a mixture of related cyclic polypeptides produced from the Tracy strain of *Bacillus subtilis.* Its high rate of cure, apparent low incidence of allergic reactions (at least relative to penicillins), and its nephrotoxicity more or less guaranteed that the antibiotic would be restricted to topical use. Bacitracin's effectiveness against gram-positive bacteria, its applicability to infections of the skin, eyes, and ears, and its lower frequency of sensitization relative to neomycin led to its enthusiastic adoption as a topical antibacterial, but the antibiotic is not without allergenic properties.

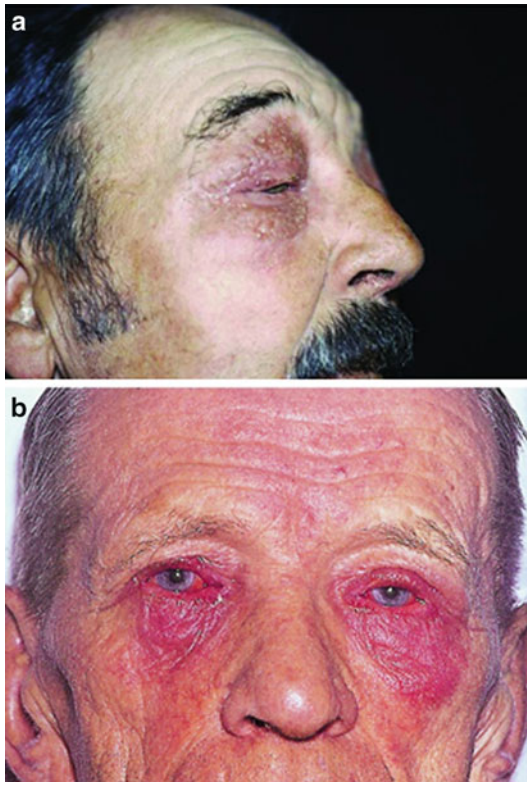


Fig. 6.7 Allergic contact dermatitis (**a**) and severe conjunctivitis (**b**) caused by neomycin eye drops (photograph courtesy of P. J. Frosch). Reproduced with permission from Brandão FM, in Contact Dermatitis, Springer-Verlag, Berlin, 2011.

6.1.5.2.1 Delayed Reactions

Bacitracin's effectiveness increases in direct proportion to its concentration, and as this and its usage rapidly increased, it soon became apparent that the drug was a significant inducer of allergic contact dermatitis. Measured incidences of bacitracin sensitivity, as low as 0.3 % in 1973, increased to a lower range estimate of 1.5 % in the last 20 years, but more recent estimates are consistently in the range 8–9.5 %. When used for conditions where wounds are open or on diseased skin such as chronic leg ulcers, the sensitization level of 24 %, although significantly less than the 34 % seen with neomycin, was still unacceptably high. Apart from patients sensitized by cutaneous application of bacitracin, others who contact the antibiotic in the course of their occupation, particularly nurses, are likely to become sensitized. Cutaneous reactions are not restricted to allergic contact dermatitis but may include localized eczema-like reactions and acute vesicular and chronic dermatitis. As with neomycin, patch tests with bacitracin should be read after a relatively long delay, usually 2–4 days after application. The drug is applied at a concentration of 20 % weight: weight in petrolatum on unbroken skin and, because bacitracin now has a well-established reputation for causing anaphylaxis, it is recommended that patients should be observed for 1 h after patches have been applied.

6.1.5.2.2 Immediate Reactions

As well as causing delayed-type IV reactions, bacitracin induces type I immediate hypersensitivities including contact urticaria and anaphylaxis. In fact, with what appears to be a constantly increasing frequency of allergic reactions to bacitracin, it has become, in a relatively short time, the topical antibiotic most recognized for eliciting anaphylaxis. Severe immediate reactions have eventuated following the application of ointments, creams, eye drops, lotions, powders, and irrigations containing the drug and the number of such cases now recorded in the literature is approaching 50. The presence of bacitracin-reactive IgE antibodies has been inferred in a number of the cases by positive patch tests and, in at least one case, presence of the antibodies was demonstrated in a fluorescent enzyme immunoassay with patient's serum by employing biotinylated bacitracin coupled to a streptavidin ImmunoCAP® (Thermo Scientific) solid phase.

6.1.5.2.3 Cross-reactions Between Bacitracin and Aminoglycoside Antibiotics

Immediate allergic reactions including anaphylaxis also occur to other aminoglycoside antibiotics with cases recorded for neomycin, gentamycin, tobramycin, framycetin, streptomycin, and dihydrostreptomycin. In some of these cases where tests were undertaken, patch and/or skin tests proved positive to the culprit aminoglycoside, but cross-reactivity with bacitracin has not been reported or, it seems, looked for.

Unexpectedly, some patients demonstrate contact allergy to both bacitracin and neomycin

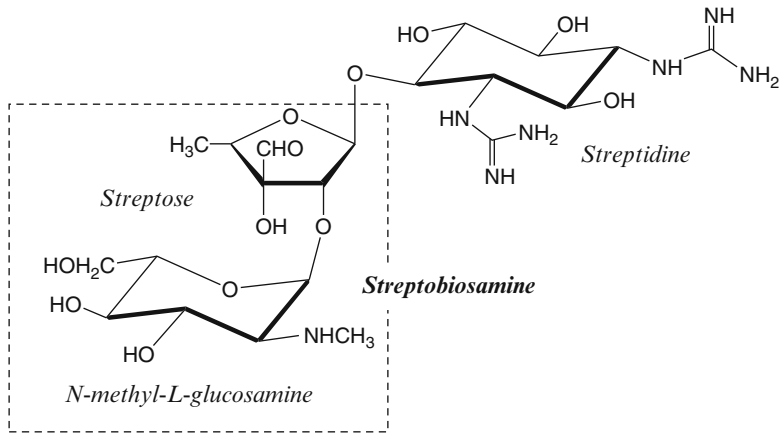
even though the two antibiotics have markedly different structures. Such co-sensitivities even extend to the cyclic peptide antibiotic polymyxin. Since structural similarities do not appear to account for this phenomenon, the explanation for the coincident reactions to all three drugs seems to lie in the similar exposure patterns both in terms of the nature of the topical exposure sites and the frequency of exposure. Reactions to bacitracin and the aminoglycoside antibiotics seem to occur particularly when the skin barrier is not intact and after prolonged use so applications in the form of ointments, creams, irrigations, and other dosage forms to open wounds, ulcers, excoriations, and skin grafts should be subject to caution. Known allergy to either bacitracin or neomycin should also preclude the use of the other antibiotics. There is a growing belief that the application of topical antibiotics to closed wounds should be strongly discouraged, that petrolatum is a suitable cost-effective protective substitute and for open wounds, and that neomycin should be avoided and bacitracin used instead although its risks should be anticipated and explained to patients.

6.1.5.3 Other Aminoglycoside Antibiotics

Emphasis here on neomycin and bacitracin in topical preparations reflects their usage over many years and this in turn has contributed to the frequency of occurrence of their now well-known adverse effects. Adverse reactions including hypersensitive responses to other aminoglycosides, namely, kanamycin, tobramycin, dihydrostreptomycin, streptomycin, framycetin, and gentamycin are also known, and again, little usage has probably influenced their reported low incidence of reactions since kanamycin and tobramycin are infrequently administered and the streptomycins are now largely withdrawn from human administration. Even though the aminoglycosides exhibit cytotoxicity, they are often formulated with the cyclic peptide antibacterials bacitracin and polymyxin for use in ear and eye preparations. An early study of medicament (including aminoglcosides)-induced contact dermatitis in patients with chronic inflammatory ear disease reached a number of important conclusions that are still relevant today. In the study group, 35 % of patients were found to have contact dermatitis to drugs used for treatment. Patch tests revealed neomycin and framycetin as the most frequent sensitizers with an incidence of 15 %, followed by gentamycin (10 %), polymyxin (5 %), and, surprising for today, bacitracin (2 %). The number of positive reactions to neomycin was probably higher than the observed incidence since tests were read after 4 rather than 7 days, and because 90 % of subjects sensitized to neomycin are also allergic to framycetin and 40 % are allergic to gentamycin, reactions to the latter two aminoglycosides were attributed to primary neomycin sensitivity. A positive skin test to gentamycin and a negative test to neomycin were, however, observed and this has been supported by more recent findings. Although the low incidence of reactions to gentamycin is often put down to its low allergenic potency, this may again simply reflect its infrequent use. A doubt commented on was the difficulty of determining whether the positive reactions to polymyxin and bacitracin were due to separate sensitizations or cross-reactions with neomycin and the conclusions from the study are still of interest today. Firstly, since 17 % of patients were allergic to neomycin or framycetin, their withdrawal from routine use was recommended; secondly, because of the high incidence of contact sensitivity to the topical antibacterials and the uncertainty that cultured organisms are both pathogenic and relevant, these topical antibiotics should be avoided; lastly, patients with persistent inflammatory disease should be investigated for drug-induced contact dermatitis and the five topical antibiotics mentioned here should be included in testing. Thirty years on from this investigation these conclusions remain relevant.

6.1.5.4 Streptomycin and a Note on Cross-sensitivity Between Aminoglycoside Antibiotics

The aminoglycoside antibiotics can be divided into two groups each made up of amino sugars linked glycosidically to an aminocyclitol which is the base streptidine in the case of streptomycin



Streptomycin

Fig. 6.8 Structure of streptomycin which is structurally and antigenically similar to neomycin (see Fig. 6.6). The aminoglycoside antibiotic streptomycin consists of the disaccharide streptobiosamine glycosidically linked to the aminocyclitol streptidine. Streptobiosamine consists of the nitrogen-containing sugar *N*-methyl-L-glucosamine and the cyclic alcohol streptose

and 2-deoxystreptamine for the other aminoglycosides considered here (see Sect. 6.1.5 and Fig. 6.6). In streptomycin, streptidine is linked to a nitrogen-containing disaccharide, streptobiosamine composed of *N*-methyl-L-glucosamine and the five-membered cyclic base alcohol streptose (Fig. 6.8). Chemically then, streptomycin and, for example, neomycin share no structural similarities and this is also true antigenically. Unlike the other aminoglycoside antibiotics considered here, streptomycin was not primarily used topically. Hypersensitivity reactions to streptomycin include maculopapular, morbilliform, erythematous and urticarial rashes, pruritus, exfoliative dermatitis, eosinophilia, stomatitis, angioedema, and anaphylactic shock. Because of its greatly reduced usage and unlike neomycin and gentamycin, allergic contact dermatitis is currently not a problem with streptomycin, but reports on anaphylaxis to the antibacterial go back over 50 years with a relatively high incidence of reactions occurring in the 1960s. However, with the decline in streptomycin therapy in humans, occasional indications of the antibiotic's allergenicity are still evident in the form of veterinary therapeutic agents, nontherapeutic contact from tiny quantities of the drug in foods and culture media, and during in vitro fertilization and immunotherapy procedures.

Reports of cross-reactions between neomycin and some other aminoglycosides, particularly gentamycin and framycetin, are well known, but, consistent with the absence of common antigenic structures between these molecules and streptomycin (compare Figs. 6.6 and 6.8), allergenic cross-sensitization has not been observed between the two different aminoglycoside groups.

Antibiotics discussed in the following sections are little, or only occasionally used and/or poorly allergenic although clindamycin, because of its broad spectrum and suitability as a satisfactory alternative for patients allergic to penicillins and cephalosporins, is administered more frequently than the others.

6.1.6 Ribostamycin

Ribostamycin is a broad spectrum aminoglycoside antibiotic isolated from *Streptomyces ribosifidicus* often used by intramuscular injection, particularly in some Asian countries where it is administered to treat pelvic inflammatory

disease. After a third injection of the drug, a female patient developed shortness of breath, flushing, and generalized pruritus, all of which spontaneously resolved. After a fourth injection of ribostamycin, epinephrine, hydrocortisine, diphenhydramine, and salbutamol were required to reverse the resultant severe hypotension, angioedema, dyspnea, dizziness, and generalized urticaria. Prick and intradermal tests revealed positive results with ribostamycin 1 mg/ml but negative responses to two other aminoglycosides tobramycin and micronomicin, suggesting immunoglobulin E-mediated hypersensitivity. A case of erythroderma following intramuscular ribostamycin revealed an interesting cross-reaction with neomycin. Patch tests were positive to both ribostamycin and neomycin, suggesting that the three-ring identity the two aminoglycosides share was responsible for the cross-reaction. Ribostamycin shares the neamine structure composed of neosamine and 2-deoxystreptamine and the ribose ring with neomycin but lacks the neosamine ring which is part of the neobiosamine structure in neomycin (see Fig. 6.6). These findings suggest the possibility of a potential hazard if a systemic aminoglycoside is given to a patient sensitized by a topical aminoglycoside such as neomycin.

6.1.7 Chloramphenicol

Originally from the bacterium *Streptomyces venezuelae*, chloramphenicol shows a broad spectrum of antimicrobial activity and, despite being widely and frequently used for a number of years following its introduction in 1949, the antibiotic has provoked a remarkably small number of hypersensitive reactions. This is reflected in the infrequency of references to the drug in both broad surveys of drug-induced adverse reactions and in the tiny number of individual case reports. In recent years, administrations of the drug have declined due to the fear of resistant organisms and some safety concerns. The most serious adverse reactions are aplastic anemia, bone marrow suppression, and rarely, anaphylaxis for which there appears to have been up to about a dozen reported cases, usually after topical application. Chloramphenicol is still prescribed as eye drops and eye ointment for the treatment of bacterial conjunctivitis because of its broad spectrum, low corneal toxicity, its property of providing therapeutic levels in aqueous humor, and its availability in preservative-free dosage forms. It is from its topical use that sensitization and hypersensitivity reactions occasionally occur including urticaria, angioedema, contact dermatitis, and, as mentioned, anaphylaxis. In the face of its declining usage, especially in the developed nations, chloramphenicol has retained its worth as a treatment for meningitis and this value is most apparent in meningitis patients with penicillin and cephalosporin allergy. In what first appeared to be an unusual but intriguing finding, cross-reactivity between dinitrochlorobenzene and chloramphenicol was reported in 40 % of patients studied for contact sensitivities. Since the molecule of chloramphenicol contains both a nitrobenzene group and two terminally linked chlorine atoms, this claim may have been given some initial credence. Follow-up investigations found no positive patch tests to chloramphenicol ointment 1 % in 100 patients with dinitrochlorobenzene sensitivity and none of 15 patients with eczema and a delayed-type reaction to chloramphenicol cross-reacted with dinitrochlorobenzene in acetone or petrolatum. An additional 27 patients primarily sensitized to dinitrochlorobenzene also showed no reaction to chloramphenicol or its salts. Misinterpretation of a primary irritant reaction as an allergic contact reaction was suggested as the explanation for the originally reported industrial chemical–antibiotic cross-reaction.

6.1.8 Clindamycin

Clindamycin is formed by substituting a chlorine atom for the 7-hydroxy substituent of the naturally occurring lincosamide antibiotic lincomycin (Fig. 6.9). Both compounds contain the unusual sulfur-containing sugar moiety methylthiolincosamide. Clindamycin is used to treat anaerobes, protozoa, and some methicillin resistant *S. aureus* infections and is applied topically

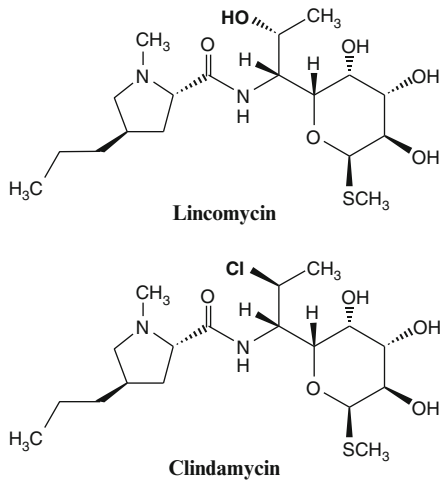


Fig. 6.9 Structures of the naturally occurring antibiotic lincomycin, which contains the sulfur-containing sugar methylthiolincosamide, and the closely related clindamycin in which the 7-hydroxy substituent is replaced by a chlorine atom

for acne. Although it is a valuable anti-infection agent especially as a substitute for the β-lactam antibiotics, clindamycin demonstrates some toxicity, particularly pseudomembranous colitis, and occasionally elicits some hypersensitivity responses. Delayed-type cutaneous reactions to clindamycin including pruritus, exanthematous rash, generalized maculopapular exanthema, erythroderma, generalized exanthematous pustulosis, and Stevens–Johnson syndrome have been reported and contact dermatitis may occur after topical application. However, to retain some perspective, attention should be drawn to a 1999 hospital study in Chicago of clindamycin-induced adverse drug reactions for the period 1995–1997 which concluded that clindamycin hypersensitivity appears to be rare with an incidence of adverse reactions lower than reported 25 years earlier. Anaphylaxis to the drug is said to occur in less than 0.1 % of treated patients, and with a literature search revealing only two cases, the first apparently in 1977, this reported incidence may also be an overestimate.

Diagnostic tests for clindamycin-induced hypersensitivities have not been widely employed and validated. No clindamycin-specific IgE antibodies have been found nor have any allergenic determinant structures been identified. Skin tests are not standardized and the optimum concentration(s) of drug to use have yet to be determined and agreed upon. For skin prick testing, a concentration of 150 mg/ml was used to detect positive reactions, and for patch tests, a concentration of 10 % in petrolatum. Intradermal testing has been employed in the concentration range 0.15–1,500 μg/ml, but its use has been limited. Diagnosis of clindamycin hypersensitivity often relies on temporal associations with drug-induced signs and symptoms. Some studies have concluded that skin tests are not adequate to identify suspected allergic reactions and provocation testing is a suggested superior alternative. In one study, a negative predictive value of only 68 % was calculated for prick and intradermal tests. Oral challenge has also been suggested to overcome false positive and negative skin responses to the drug. Positive skin prick and intradermal tests to clindamycin were obtained in a patient who developed erythroderma a week after receiving the antibiotic intravenously. No immediate skin responses to the tests were observed, but 12 h delayed responses were seen following skin prick tests with clindamycin at a concentration of 150 mg/ml and intradermal tests with the drug in the range 0.015–1.5 mg/ml. The same tests were negative in five control patients. Patch testing with clindamycin 10 % in petrolatum yielded positive responses in patients with delayed cutaneous adverse reactions in the form of generalized maculopapular exanthema following administration of the antibiotic. No positives were detected in control subjects, but as with skin prick and intradermal tests, patch testing with clindamycin needs standardization and validation studies. Patch tests using a suspension of ground clindamycin tablet in saline (150 mg/ml) and prick tests with a parenteral solution of the drug (150 mg/ml) were performed on 33 patients with a history of skin reactions in temporal association with treatment with clindamycin. To exclude IgE antibody-mediated hypersensitivity, prick tests were read after 20 min. Patch tests were removed after 1 day and late reactions were assessed after 2, 3, and 4 days. In four of the patients, matching positive delayed reactions were seen with both

skin tests and patch testing identified an additional positive reactor. Challenge tests elicited rashes in a further six patients—three patients with exanthema, two with symmetrical drug-related intertriginous and flexural exanthema, and one with a non-pigmented fixed drug eruption. The investigators concluded that combined skin tests plus challenge testing are necessary to rule out allergic clindamycin hypersensitivity.

6.1.9 Pristinamycin

Pristinamycin is a member of the streptogramin class of antibiotics effective against vancomycin-resistant *S. aureus* and vancomycin-resistant enterococcus. It is biosynthesized by the bacterium *Streptomyces pristinaespiralis* and consists of two components, pristinamycin IA (a macrolide) and the depsipeptide pristinamycin IIA (also known as streptogramin A) in the ratio of 30:70. Streptogramins, also called synergistins, are made up of two groups, streptogramins A and B.

There are at least four documented cases of anaphylaxis to the drug, but hypersensitivity reactions are otherwise rare, usually cutaneous, and of the delayed type. In one study of 29 patients with cutaneous reactions to pristinamycin, maculopapular rash occurred in 18, erythroderma in 9, angioedema in 1, and Stevens–Johnson syndrome in 1 patient. The patients' skin test responses were assessed to pristinamycin, and related streptogramins, quinupristin/dalfopristin and virginiamycin, were also tested on some patients to assess cross-reactions between the drugs. Patch and intradermal but not prick tests proved to be of diagnostic value and detection of some positive reactions to quinupristin/dalfopristin and virginiamycin as well as to pristinamycin led to the conclusion that cross-reactivity existed between the drugs and therefore all streptogramins should be avoided in patients with adverse cutaneous reactions to pristinamycin.

6.1.10 Fosfomycin

Originally called phosphonomycin, fosfomycin is a broad spectrum antibiotic produced by a bacterium of the *Streptomyces* genus. The structure, a methyloxirane derivative of phosphonic acid, is quite unlike any of the other antibiotics. Fosfomycin shows promise for the treatment of multidrug-resistant Enterobacteriaceae infections including extended spectrum β-lactamase-producing organisms and is administered, usually as a single large dose, for infections of the urinary tract. At least three cases of anaphylactic shock to the antibiotic, two recently to fosfomycin tromethamine, have been reported, but there appears to be no other reports of adverse reactions presumably reflecting the paucity of clinical research, infrequent usage of the drug, and/or a low incidence of reactions.

6.2 Antimicrobials Other than Antibiotics

6.2.1 Sulfonamides

As already stated, sulfonamides used as chemotherapeutic agents to treat infections will be referred to here as antimicrobials (or antibacterials) not antibiotics. Other drugs with a sulfonamide structure but no antibacterial action will be termed, for example, a sulfonamide diuretic (like furosemide and hydrochlorothiazide) or a sulfonamide rheumatologic agent (such as sulfasalazine).

6.2.1.1 Structure–Activity Considerations

A sulfonamide contains a sulfonyl group connected to an amine and has the general formula $R_1SO_2NR_2R_3$. In everyday clinical medicine and amongst the public, a sulfonamide used to treat infections is often called a "sulfa drug". Chemically, for the antibacterial drugs, the generic name sulfonamide refers to derivatives of *para*-aminobenzenesulfonamide or sulfanilamide, a structural analog of *para*-aminobenzoic acid (Fig. 6.10). Structural prerequisites for antibacterial action are, to greater or lesser extent, reflected in the structural features of sulfanilamide. The arylamine group at the N^4 position of the sulfonamide structure must be *para* to the position of direct attachment of the sulfur to the

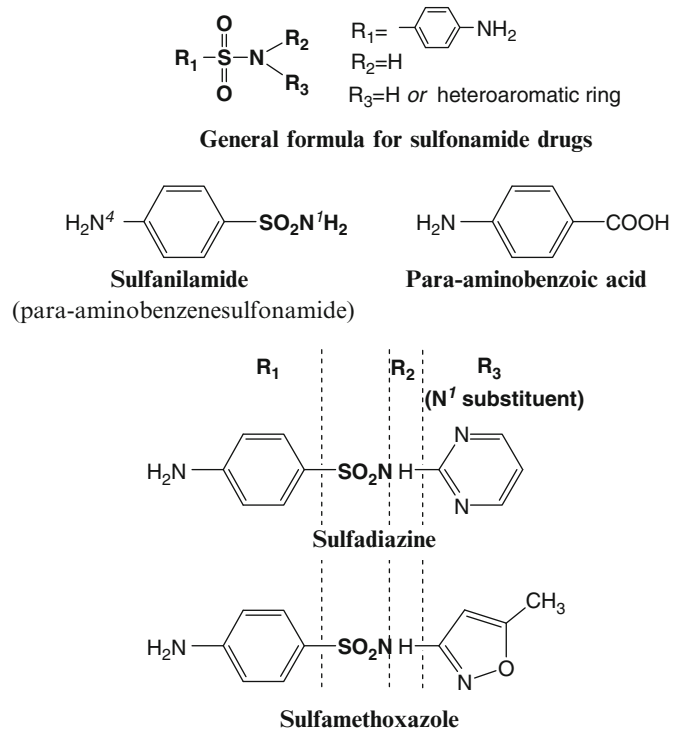


Fig. 6.10 General structure of a sulfonamide antibacterial drug, general formula $R_1SO_2NR_2R_3$, containing a sulfonyl group connected to an amine. Sulfonamide antibacterials are derivatives of *para*-aminobenzenesulfonamide or sulfanilamide, a structural analog of *para*-aminobenzoic acid. For antibacterial action, the arylamine group at the N^4 position of the sulfonamide structure must be *para* to the position of direct attachment of the sulfur to the benzene ring. The amine must be unsubstituted or in a form that can be converted back to the free amine. The nature of the attached group at N^1 (R_3 substituent) strongly influences the antibacterial activity of the molecule. For example, attachment of the methyl substituted isoxazolyl group at N^1 as in sulfamethoxazole produces a sulfonamide with marked antibacterial action

benzene ring and, as in *para*-aminobenzoic acid, the amine must be unsubstituted or at least in a form that can be converted in the tissues back to the free amine (Fig. 6.10). In general, the addition of other substituents to the benzene ring or replacement of the ring with another ring leads to a loss of antibacterial activity. The $–SO_2NH_2$ group attached directly to the ring is essential and not only is substitution at the N^1 nitrogen essential, but the nature of the attached group strongly influences the antibacterial activity of the molecule. The sulfonamide antibacterials, by virtue of their similarity in chemical structure to *para*-aminobenzoic acid, competitively inhibit the enzyme-assisted incorporation of this essential metabolite into dihydropteroic acid, the immediate precursor of folic acid. Hence, microorganisms that must synthesize their own folic acid are sensitive to sulfonamide antibacterials. Substitution of aromatic heterocyclic groups at N^1, for example, a diazine nucleus in sulfadiazine (Fig. 6.10), often produces sulfonamides of higher potency (for example, compared to sulfanilamide) even though such heterocyclic derivatives are chemically less like the metabolite *para*-aminobenzoic acid than sulfanilamide. Ionization and dissociation measurements seem to provide an explanation for this. It seems that the addition of such weakly basic substituents to the $–SO_2NH_2$ group changes the electrical properties of the modified group, that is, the $–SO_2NHR$ group, in such a way that its properties become more like the carboxylic acid group of *para*-aminobenzoic acid.

6.2.1.2 Hypersensitivity Reactions

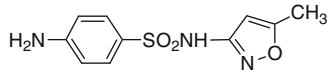
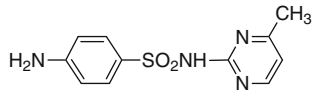
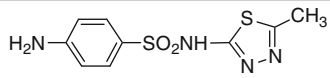
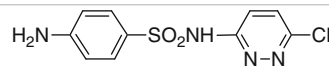
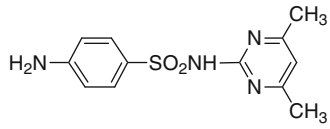
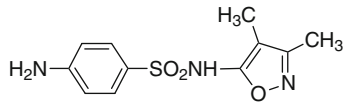
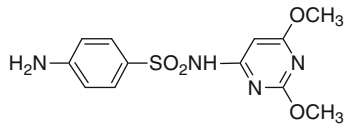
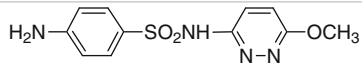
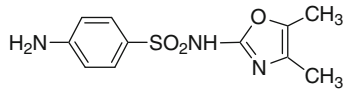
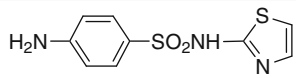
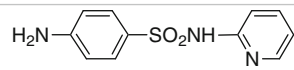
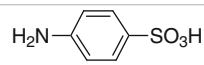
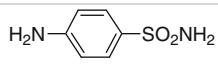
The overall incidence of adverse reactions to sulfonamide antibacterials is about 3–5 %. Reactions are many and varied and may involve almost every organ system of the body including the blood, bone marrow, liver, kidney, skin, and peripheral nerves. Individual adverse responses include nausea, vomiting and anorexia, hemolytic anemia, aplastic anemia, agranulocytosis, thrombocytopenia, eosinophilia, renal damage due to crystalluria (with older sulfonamides), hepatitis, goiter, hyperthyroidism, rarely peripheral neuritis, and hypersensitivity reactions. The latter are said to occur in about 3 % of courses of the drug and in approximately 50–60 % of sulfonamide-treated patients with human immunodeficiency virus (HIV) infection. Many of the reactions involve the skin and mucous membranes. A variety of more severe reactions may also occur including potentially lethal toxidermias and a delayed hypersensitivity-type syndrome characterized by fever, skin rash, and multi-organ toxicity. All of the above reactions are distinct from the type I true allergic reactions which are immediate in onset, usually non-febrile, mediated by drug-reactive IgE antibodies, and may be accompanied by urticaria and symptoms of anaphylaxis including wheezing, shortness of breath, hypotension, angioedema, bronchospasm, and ultimately cardiovascular collapse. The involvement of sulfonamide antibacterials in these hypersensitivities of the immediate type will be dealt with first.

6.2.1.2.1 Type I, IgE Antibody-Mediated Reactions to Sulfonamide Antimicrobials

These are the best worked out and defined sulfonamide-induced hypersensitivity reactions with the best defined allergenic drug structures. Patients with symptoms characteristic of an immediate type I reaction such as generalized itch, urticaria, angioedema, wheezing, and other anaphylactic-like symptoms following ingestion of standard oral doses of co-trimoxazole (trimethoprim 80 mg and sulfamethoxazole 400 mg per tablet) were investigated and the investigative procedures undertaken and the results obtained are provided as a general protocol for studying a drug-induced immediate allergic reaction. A detailed analysis of the structure–activity findings in the form of comparative inhibition results obtained with selected analogs is provided as a guide for how quantitative hapten inhibition studies can be used to define the allergenic fine structural features of a drug, that is, the structural features most complementary to the induced IgE antibody combining sites. Put more simply, this means the structural features responsible for the "goodness of fit" between a drug and its complementary antibodies. Interpretations of the results are reproduced from the author's studies with permission.

Skin prick testing was carried out using Septrin Parenteral Infusion® (Wellcome) containing 16 mg/ml of trimethoprim and 80 mg/ml of sulfamethoxazole. For intradermal testing, trimethoprim and sulfamethoxazole were dissolved in the minimum quantity of 0.1 M sodium hydroxide and benzyl alcohol, respectively, before diluting with physiological saline to a dilution of 1 mg/ml. Physiological saline solutions containing the same amounts of sodium hydroxide or benzyl alcohol were used as controls. Tests were performed by injecting 0.02 ml of test or control solution and generally testing was commenced with the test solutions diluted 1:100. Sera from patients with a history of an immediate response and/or a positive skin test to sulfamethoxazole were examined in vitro in radioimmunoassays using a sulfamethoxazole–Sepharose solid phase covalent complex and an ^{125}I-labeled anti-human IgE antibody as second antibody to detect binding of patients' serum IgE antibodies to the immobilized drug. Sera from normal, healthy subjects, cord blood, and patients highly allergic to mites and pollens were used as controls. As an additional control, no significant binding was observed when the patients' sera were tested with ethanolamine–Sepharose and Sepharose alone as control solid phases. To check on the absolute specificity of antibody binding, quantitative hapten inhibition experiments were carried out using a range of sulfonamide analogs. In the typical example shown (Table 6.2), easily the most potent inhibitors were sulfamethoxazole and sulfamerazine, the former requiring half the

Table 6.2 Inhibition by antibacterial sulfonamides of the binding to a sulfamethoxazole-solid phase[a] of IgE antibodies in the serum of a patient[b] allergic to sulfamethoxazole

Compound	Structure	Amount (nmol/tube) of sulfonamide for 50 % inhibition of binding of IgE antibodies	Inhibition (%) of binding to sulfamethoxazole–Sepharose by 1 μmol/tube of compound
Sulfamethoxazole		265	66
Sulfamerazine		540	62
Sulfamethizole		>1,000	39
Sulfachloropyridazine		>1,000	46
Sulfamethazine		840	52
Sulfisoxazole		>1,000	40
Sulfadimethoxine		900	51
Sulfamethoxypyridazine		>1,000	31
Sulfamoxole		>1,000	33
Sulfathiazole		>1,000	42
Sulfapyridine		>1,000	37
Sulfanilic acid		>1,000	2
Sulfanilamide		>1,000	14

[a]Sulfamethoxazole–Sepharose covalent complex used in radioimmunoassay with a ^{125}I-labeled second antibody
[b]Patient experienced severe itch and rash to Septrin® (two tablets) 1 year after experiencing an anaphylactic-like reaction to Septrin® tablets (trimethoprim 80 mg and sulfamethoxazole 400 mg)
Adapted from Harle DG et al. Mol Immunol 1988; 25:1347 with permission

amount of drug as the latter to achieve 50 % inhibition, followed by sulfamethazine. Ten other analogs were far less well recognized with, in general, 600–900 nmol per tube of drug needed for just 30–40 % inhibition. Sulfanilic acid and sulfanilamide, compounds without a heterocyclic ring attached at the N^1 position, were virtually inactive producing only 2 and 14 % inhibition, respectively, at 1,000 nmol per tube. Close comparisons of the structures of the sulfonamides examined and the corresponding inhibitory values revealed clearly that compounds with one methyl substituent on a five- or six-membered aromatic heterocyclic ring containing at least one nitrogen atom immediately adjacent to the point of attachment to the N^1 sulfonamido nitrogen were the structures most complementary to the IgE antibody combining sites (Table 6.2). The structures of sulfamethoxazole and sulfamerazine show a 5-methyl-3-isoxazolyl N^1 substituent in the former and a 4-methyl-2-pyrimidinyl group in the latter compound. Sulfamethazine, or *N′*-(4,6-dimethyl-2-pyrimidinyl)sulfanilamide, the dimethyl derivative of sulfamerazine was the third best inhibitor. Each of the three best inhibitors has a methyl group on the carbon β to the sulfonamido substituent and either a five- or a six-membered heterocyclic ring (Fig. 6.11). The methyl group and the position of the nitrogen atom are common to all three structures and these features are clearly important for recognition by the complementary IgE antibodies. The fact that sulfamethazine, the dimethyl derivative of sulfamerazine, proved such a relatively poor inhibitor suggested that only one methyl group is necessary for recognition. This is further supported by results obtained with the close analog of sulfamethoxazole, sulfamoxole which contains a five-membered 4,5-dimethyl-2-oxazolyl ring with the oxygen atom adjacent to the point of attachment of the ring to the sulfonamido nitrogen atom (Table 6.2). Unlike sulfamethoxazole, sulfamoxole contains two methyl groups at the four and five positions of the heterocyclic ring and the lower inhibitory potency of sulfamoxole can probably be explained by the presence of the additional methyl group at position four of the ring which may sterically hinder the binding of the IgE antibodies particularly to the methyl group linked to position five. The relative bulk and close proximity of the methyl groups are readily seen in the model shown in Fig. 6.11.

Each of the above considerations, together with the finding that sulfanilamide was virtually without inhibitory effect, led to the overall conclusion that the 5-methyl-3-isoxazolyl group on the sulfamethoxazole molecule is the allergenic determinant with the methyl substituent on the ring being a dominant fine structural feature of the determinant.

6.2.1.2.2 Delayed Onset Sulfonamide Hypersensitivity Reactions

These reactions, characterized by fever, a morbilliform or maculopapular non-urticarial skin rash, occasionally multi-organ toxicity including the liver and blood, and often eosinophilia, generally occur 1–2 weeks after the initiation of therapy. Rare patients may progress to life-threatening reactions such as Stevens–Johnson syndrome and toxic epidermal necrolysis. The number of these lethal reactions to all drugs totals about ten cases per million persons per year with the incidence of sulfonamide-induced reactions being one of the highest. All drug-induced hypersensitivity reactions must occur via direct toxic effects on tissues or via immune processes, so, bearing in mind the multitude of effects seen in these non-type I, delayed hypersensitivities, it seems that the involvement of any one or more of types II, III, or IV as well as direct cytotoxic effects of the drug are likely or possible in provoking reactions. Other possibilities are direct cytotoxic effects or the induction of an immune response by the drug or its metabolite(s) bound to an endogenous protein, probably of cellular origin, or the parent unmetabolized sulfonamide might interact directly with antibodies and/or T cells.

Current thinking and most research efforts are based on the belief that the pathogenesis of the reactions involves bioactivation of the sulfonamide, forming reactive metabolites that act as the initial reactive agents in a sequence of interactions that ultimately lead to the patients'

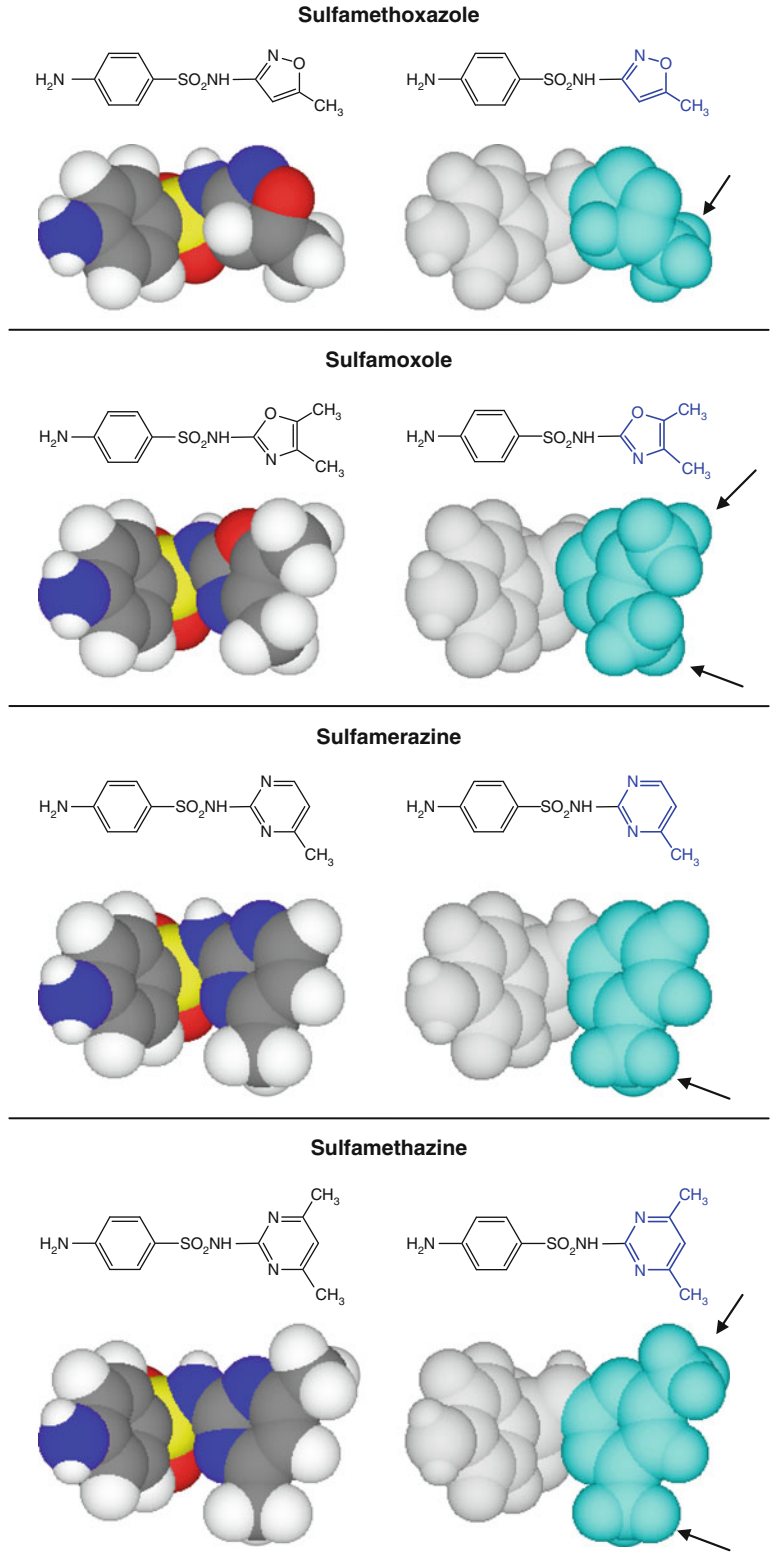


Fig. 6.11 Two-dimensional and three-dimensional CPK space-filling molecular models of sulfamethoxazole and three allergenically cross-reactive sulfonamides, sulfamoxole, sulfamerazine, and sulfamethazine. Important structural features identified for cross-reactivity are an N^1 aromatic heterocyclic ring containing at least one nitrogen and with a methyl substituent (*arrowed*) β to the point of attachment of the ring to the nitrogen (see also Table 6.2)

adverse responses. Metabolism of sulfonamide antimicrobial drugs proceeds via a number of processes. In humans, the largest proportion of an administered sulfonamide, for example, sulfamethoxazole, is metabolized in the liver by *N*-acetyltransferase to the N^4 acetylated form that is nontoxic and eliminated in the urine while about 15 % of the drug is glucuronidated at the N^1 nitrogen and also renally excreted. Glucuronidation can occur at the N^4 nitrogen but, unlike the N^1 metabolite, the product is unstable. A small fraction of sulfamethoxazole, about 10 %, is metabolized to a reactive hydroxylamine intermediate by several enzymes, particularly CYP2C9 (cytochrome P450), myeloperoxidase (MPO), and perhaps cyclooxygenase (COX), to the unstable hydroxylamine intermediate that auto-oxidizes to the highly reactive nitroso derivative nitroso-sulfamethoxazole (Fig. 6.12). Although cyclooxygenase was thought to be involved in the metabolic conversion to the hydroxylamine, that is now in doubt. The hydroxylamine derivative is thought by some to be involved in a number of the adverse reactions including hepatitis, nephritis, thrombocytopenia, lupus erythematosus, and the sulfonamide hypersensitivity syndrome. Nitroso-sulfamethoxazole can itself be acetylated and eliminated or it can react with glutathione and be reduced back to the hydroxylamine derivative. Nitroso-sulfamethoxazole, but not the parent drug, can react covalently with cysteine residues of cellular surface proteins including skin keratinocytes, circulating peripheral blood mononuclear cells and splenocytes, and serum proteins, predominantly immunoglobulins and albumin, to form hapten–protein complexes. In the latter case, reaction probably proceeds via Cys34 which is highly reactive with electrophiles. The ultimate drug determinant involved in the sulfonamide-induced delayed hypersensitivity reaction is believed to be the sulfonamide hapten made antigenic by linkage to carrier proteins but, from this point on, the question becomes, what specific immunological processes are involved in the pathogenesis of the reaction? A little more than a decade ago some investigators pointed out that drug-reactive antibodies and/or T cells had rarely been demonstrated in patients with sulfonamide hypersensitivity. It now seems accepted that T cells are involved although the nature of the antigen(s) reacting with the specific T cell receptor remains incompletely defined. The precise mechanisms underlying the hypersensitivity/adverse reactions in the patients are also yet to be worked out in detail, although again T cells are thought to be involved since hapten–protein antigens stimulate CD4(+) regulatory and CD8(+) effector T cells from hypersensitive patients. In drug hypersensitivity research where bioactivation of the drug is involved, the relationship of drug metabolism to the immune response has usually been obscure. Recent results suggest that antigen-presenting cells alone are sufficient to generate metabolites of sulfamethoxazole, produce the hapten–protein antigen complexes, and ultimately induce the T cell response to the drug. Dendritic cells are thought to activate sulfamethoxazole intracellularly to nitroso-sulfamethoxazole which is not only chemically more reactive but also more immunogenic/allergenic. Stimulation of human T cells with sulfamethoxazole, and with its nitroso and hydroxylamine metabolites, generated T cell clones that showed three recognition patterns: 14 % were sulfamethoxazole-specific, 44 % were sulfamethoxazole metabolite-specific, and 43 % were stimulated with sulfamethoxazole and its metabolites. Although the sulfamethoxazole-responsive clones were specific for the stimulating sulfonamide, a large proportion of nitroso-sulfamethoxazole-specific clones also responded to nitroso metabolites of sulfadiazine and sulfapyridine but not nitrosobenzene, that is, T cell responses can occur via cross-reactivity with the haptenic immunogen.

The complexity of the findings and some interpretations in the large body of research on sulfonamide hypersensitivity involving the enzyme-induced generation of drug metabolites; unstable intermediates; protein-reactive species; the formation of cell surface and intracellular protein adducts; co-stimulatory signaling; and the development of an antigen-specific T cell response leave some doubts and questions. For

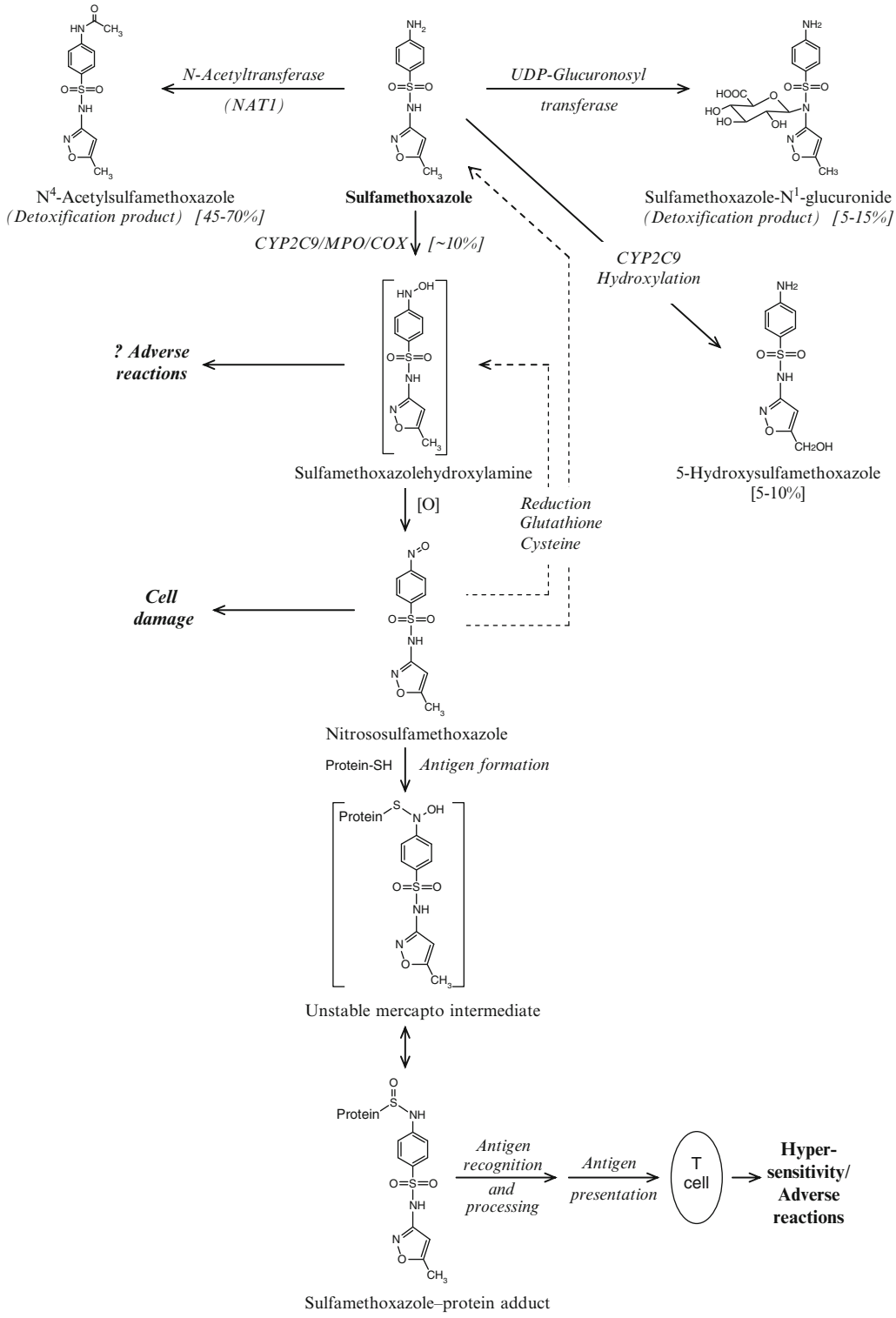


Fig. 6.12 Bioactivation of sulfamethoxazole to its reactive metabolite and subsequent steps leading to the metabolite-specific immune response and hypersensitivity/tissue damage

example, the disparity of some results in laboratory animals and humans produces doubts about the relevance of the laboratory findings nor is it clear why apparently all sulfamethoxazole-allergic patients show T cell responses to both the nitroso derivative of sulfamethoxazole and the parent drug. Attempted obscure explanations involving the amorphous concept of "avidity spreading" to the continually present parent drug do nothing to aid understanding. One is still left with the question of individual susceptibilities. Sulfamethoxazole induces hypersensitivity reactions in 1–3 % of those exposed to it (and in 50 % of HIV patients). Every patient given the drug is exposed to it and its nitroso metabolite so why don't all patients develop a hypersensitivity reaction to the antigen formed by the metabolite reacting with protein? Possible explanations advanced to account for different susceptibilities of individuals include patients with a slow acetylator phenotype that reduces their capacity to detoxify reactive metabolites; glutathione polymorphisms; the need for what has vaguely been described as "co-stimulation" for T cell receptor activation; and the possible need for complementary bidirectional drug binding domains within the major histocompatibility complex (MHC) and the T cell receptor. The latter possible explanations add an even more bewildering layer of complexity on an already densely complex narrative.

An apparent high risk of sulfonamide hypersensitivity in patients with hematological malignancies was the stimulus for a recent study designed to look for deficiencies in sulfonamide detoxification pathways. Patients were examined for levels of glutathione, ascorbate, cytochrome b_5, and cytochrome b_5 reductase, but no deficiencies of the blood antioxidants and cytochromes were found. In a secondary study, the incidence of drug hypersensitivity following intermittent trimethoprim–sulfamethoxazole prophylaxis was compared to the incidence reported for high dose regimens. After 3–4 weeks of the administration of trimethoprim–sulfamethoxazole 960 mg three to four times weekly to 22 patients, no patient developed sulfamethoxazole-specific T cells and only one patient developed a rash.

6.2.1.3 Reactions to Sulfamethoxazole in Patients Infected with Human Immunodeficiency Virus

With the exception of sulfamethoxazole (generally in combination with trimethoprim), sulfonamides are no longer heavily and widely used. However, the drug, again with trimethoprim, has found specialized application due to its effectiveness against *Pneumocystis jirovecii* in immunocompromised HIV-infected patients with *Pneumocystis* pneumonia (PCP) and other potentially life-threatening opportunistic infections. For effective prophylaxis of PCP, the drug combination is given as a single double-strength daily dose of 160 mg of trimethoprim and 800 mg of sulfamethoxazole. A serious drawback to the use of these drugs in HIV-infected patients is a high rate of adverse reactions that can range up to 50–60 % and require discontinuation of therapy. The withdrawal of the drugs is lower during dosage for prophylaxis than during treatment, but patients receiving secondary prophylaxis may experience reactions despite successful previous acute treatment. For patients with a history of reactions, rechallenge provoked adverse reactions in 13–47 % of subjects, rates that are similar to the incidences of reactions in patients with primary adverse reactions. Regardless of previous reaction history, adverse reactions are similar and include rash, fever, flu-like symptoms, and gastrointestinal disturbances. In two early trials, reactions to high doses of sulfamethoxazole–trimethoprim resolved in more than 80 % of patients despite the therapy continuing. Because of the efficacy of sulfamethoxazole–trimethoprim for PCP prophylaxis, the reintroduction of the drug combination in patients who had a prior adverse reaction is sometimes desired. With this in mind, a randomized, controlled trial was undertaken to compare dose escalation with direct rechallenge regimens. With the primary end point being the patient's tolerance of single-strength sulfamethoxazole–trimethoprim for 6 months, 75 % of the dose-escalation group and 57 % of the direct rechallenge group were able to receive the single-strength dosage for the selected time, demonstrating that it is possible to successfully

reintroduction the drugs to a high proportion of HIV-infected patients who previously experienced treatment-limiting adverse reactions.

The rate of drug-induced adverse reactions in HIV-infected patients is over five times higher than the rate for HIV-negative subjects, and although the reason for this is not known, some observations related to reduction of the reactive nitroso-sulfamethoxazole metabolite may be pertinent. Deficiencies of ascorbate and thiols (for example glutathione), two agents that effect reduction of the metabolite in vivo, have been reported in HIV-infected subjects and it has been speculated that this may result in increased metabolite-mediated lymphocyte toxicity and a significantly increased risk of hypersensitivity reactions.

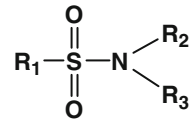
An unusual acute anaphylactic-like syndrome that resembles septic shock has been observed in some HIV-infected patients following the administration of co-trimoxazole. The severe systemic reaction is characterized by fever, hypotension, and pulmonary infiltrates, but absence of bronchospasm and laryngeal edema are points of difference with classic anaphylaxis. Because of the similarities between this hypotensive syndrome and septic shock, it has been suggested that tumor necrosis factor (TNF), a mediator of septic shock, is released during episodes of the syndrome. However, TNF and IgE antibodies to co-trimoxazole were not detected and there was no depression of complement in a patient who responded with a second episode of shock after being rechallenged with the drug combination.

A number of trials have confirmed the efficacy of desensitization with trimethoprim–sulfamethoxazole in HIV-infected patients. Successful desensitizations, both rapid and slow, were achieved with success rates often in the 70–100 % range depending on the number of patients involved and the CD4+ and CD8+ counts. Desensitization seemed more often successful with lower CD4+ percentages and CD4+:CD8+ ratios.

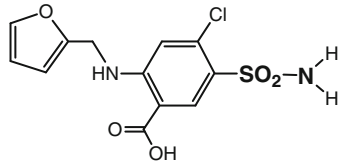
Polymorphisms in genes involved in sulfamethoxazole biosynthesis, metabolite detoxification, and the regulation of glutathione levels are of interest in attempts to understand the risk of hypersensitivity induced by the drug. In HIV/AIDS patients, glutathione levels are progressively depleted and this parallels the higher incidence of sulfamethoxazole-induced hypersensitivity in these patients. A polymorphism in glutamate cysteine ligase catalytic subunit (GCLC), also known as gamma-glutamylcysteine synthetase, the rate-limiting enzyme of glutathione synthesis, was recently found to be associated with sulfamethoxazole-induced hypersensitivity in HIV/AIDS patients. A single nucleotide polymorphism (SNP), reference SNP number rs761142 T>G, in GCLC was significantly associated with reduced mRNA expression in liver and B lymphocytes and with the drug hypersensitivity. This finding was interpreted as support for the role of reactive metabolites in the pathogenesis of sulfamethoxazole-induced hypersensitivity and led to the speculation that GCLC may be associated with reactions caused by some other drugs.

6.2.1.4 The Question of Cross-reactivity Between Antimicrobial and Non-antimicrobial Sulfonamides

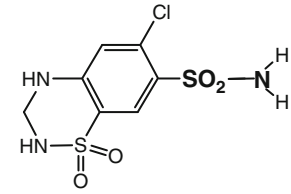
Chemically, a sulfonamide is a compound that contains a sulfonyl group connected to an amine and which has the general formula shown in Fig. 6.13 (see also Fig. 6.10). Besides the sulfonamide antimicrobials, many drugs of diverse pharmacological action used in medicine today are sulfonamides. Some that are widely used include the diuretics furosemide and hydrochlorothiazide; sulfonylureas such as glyburide and tolbutamide; uricosurics, e.g., probenecid; sulfasalazine used as a rheumatologic agent and for inflammatory bowel disease; the selective serotonin-1 receptor agonist sumatriptan; and the cyclooxygenase-2 inhibitor celecoxib (Fig. 6.13). These drugs are distinguished from the antimicrobials by the absence of an unsubstituted arylamine group (or a group capable of being converted to the free amine) attached directly to the benzene ring at the N^4 position of the antimicrobial compounds (compare Fig. 6.10). Another important difference is the presence in the sulfonamide antimicrobials of an aromatic five- or six-membered heterocyclic ring (except for the "parent" antimicrobial sulfanilamide), generally containing at least one nitro-



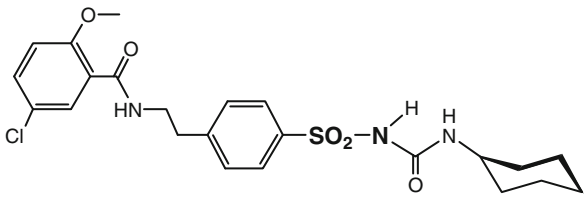
Sulfonamide general formula



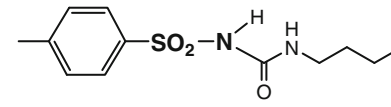
Furosemide (Frusemide)



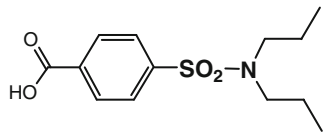
Hydrochlorothiazide



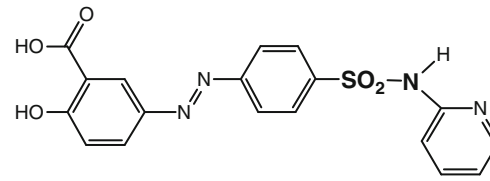
Glyburide (Glibenclamide)



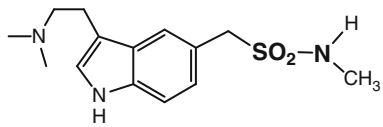
Tolbutamide



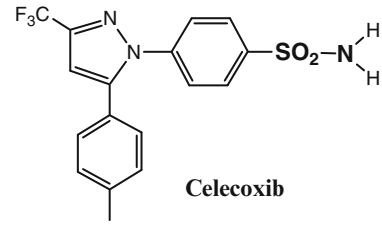
Probenecid



Sulfasalazine



Sumatriptan



Celecoxib

Fig. 6.13 Examples of some non-antimicrobial sulfonamides containing a sulfonyl group connected to an amine but which are not recognized by sulfamethoxazole-reactive IgE antibodies. Note the absence of both an unsubstituted arylamine attached at N^4 (see Fig. 6.10) and (except for sulfasalazine) an aromatic five- or six-membered heterocyclic ring at N^1

gen atom, attached to the N^1 nitrogen of the sulfonamide group (Fig. 6.10, Table 6.2).

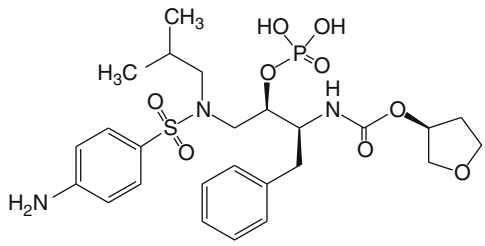
Loose usage of the term "sulfa allergy," the generally poor understanding of the mechanism of sulfonamide-related adverse reactions, and some reports of patients reacting to non-antimicrobial sulfonamides have sometimes led to a situation where all sulfonamides, regardless of chemical structure, are considered contraindicated in patients with a history of so-called "sulfa allergy." The presence of the sulfonamide group in such a wide variety of frequently used drugs raises the question of possible allergenic cross-reactivity between any, or perhaps even some, drugs because of a common sulfonamide group. In immediate hypersensitivity reactions to antimicrobial sulfonamides, IgE antibody-mediated cross-reactivity may occur between drugs in this group and cross-reactivity has been demonstrated in some delayed reactions, so is the presence of a common sulfonamide structure in different non-antimicrobial drugs also recognized in some immediate and delayed reactions? This does not seem likely since compounds such as furosemide, tolbutamide, celecoxib, and so on (Fig. 6.13) do not contain the N^1 aromatic heterocyclic ring substituent necessary for recognition by IgE antibodies and apparently necessary for adverse reactions such as hypersensitivity syndrome and lethal toxidermias to occur. However, a few case reports indicated that cross-reactions seem to occur among various sulfonamide-containing drugs, but there has been no universal acceptance of this conclusion. In one case, skin tests on a patient following an anaphylactic reaction to furosemide showed positive reactions to the culprit drug and sulfamethoxazole 0.03 mg/ml. In an attempt to rule out allergic recognition of the *para*-amino group linked to the benzene ring, an epicutaneous test with *p*-phenylenediamine and parenteral challenge with acetaminophen (paracetamol) and procaine were carried out. Both challenges were tolerated. Another example that seems to indicate cross-reactivity between a non-antimicrobial sulfonamide and sulfamethoxazole is contained in a report describing a fixed drug eruption on the lips of a patient after taking rofecoxib, a nonsteroidal anti-inflammatory drug containing the sulfonamide group. The same reaction on the patient's lips occurred 4 months later after taking rofecoxib and a challenge 1 month later with a low dose of the drug again elicited the same response. Most interesting of all was the demonstration of an identical reaction at the same location 2 weeks later when the patient was challenged orally with sulfamethoxazole–trimethoprim. Findings such as these suggested that cross-reaction between different antimicrobial and non-antimicrobial sulfonamides may occasionally occur, or are at least possible, but based on our current knowledge of the chemistry, metabolism, immune responses, and clinical findings, cross-reactions are currently thought not to occur.

In the light of this background, it is interesting and worthwhile to consider a retrospective cohort study that examined the risk of an allergic reaction within 30 days after receipt of a non-antimicrobial sulfonamide in 969 patients who had a prior allergic reaction to a sulfonamide antimicrobial and in 12,257 patients who experienced no allergic reaction after receiving a non-antimicrobial sulfonamide. Analysis showed that patients with a prior allergy to an antimicrobial sulfonamide were more likely than the nonallergic patients to react with a non-antimicrobial sulfonamide with the percentages of reactors being 9.9 and 1.6 %, respectively. These findings indicated that allergy to an antimicrobial sulfonamide *is* a risk factor for a subsequent reaction to a non-antimicrobial sulfonamide, but the risk factor is even greater for patients with a prior hypersensitivity to a sulfonamide antimicrobial who received *penicillin* (14 %) compared with patients with no prior hypersensitivity to a sulfonamide antimicrobial who received penicillin (2 %). Interestingly, in trying to get these results into some sort of overall perspective of drug allergy, the risk of an allergic reaction to a non-antimicrobial sulfonamide in patients previously allergic to a sulfonamide antimicrobial proved to be lower than the risk of a reaction to a penicillin in sulfonamide antimicrobial-allergic patients. Lastly, the risk of an allergic reaction to a non-antimicrobial sulfonamide was lower in patients with a history of sulfonamide antimicrobial hypersensitivity than in those with a history of penicillin hypersensitivity.

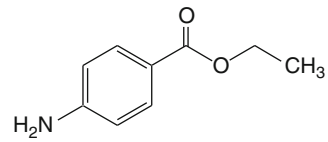
Summing up then, it seems that hypersensitivity to a sulfonamide antimicrobial is a risk for a subsequent reaction to a non-antimicrobial sulfonamide, but prior penicillin allergy is at least as great, or an even greater, risk. The most likely explanation for the conclusions of this study is that the patients showed a general predisposition to allergic reactions rather than simply allergenic cross-reactivity with drugs containing the sulfonamide group. The message for prescribers who are anxious to know whether or not there is broad cross-reactivity amongst sulfonamide drugs is simply that there is an increased risk to drugs in general, rather than just sulfonamides, in patients with a history of allergy of any type to sulfonamides or penicillins. The general message for drug allergy in the clinic is not new and has been demonstrated before with a variety of different drugs: the history of an adverse drug reaction increases the risk of a subsequent adverse drug reaction.

The Possibility of Cross-reactions with Non-antimicrobial Sulfonylarylamines and Arylamines

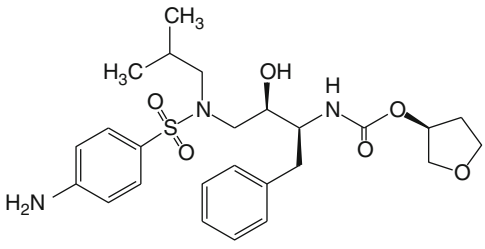
Some early research investigators concluded (in the absence of convincing and clear supporting data) that the N^4 arylamine moiety of sulfonamides like sulfamethoxazole is a major determinant recognized by IgE antibodies. This therefore raises the questions of the safety in patients allergic to antibacterial sulfonamides of non-antibacterial sulfonamides containing an unsubstituted arylamine group at the N^4 position as well as drugs with such an unsubstituted arylamine group but lacking a sulfonyl group. The sulfonylarylamine antiretrovirals amprenavir and fosamprenavir, used in the management of HIV-infected patients, contain a 4-amino benzenesulfonamido group, and although they lack the required aromatic heterocyclic ring structure attached at the N^1 nitrogen of sulfonamide antimicrobials, it seems possible, on structural grounds, that cross-reactions might occur with sulfonamide-reactive IgE antibodies and in delayed hypersensitivity responses. Fosamprenavir is a pro-drug metabolized to form amprenavir (Fig. 6.14), a protease inhibitor used to treat HIV infection. Although there are some references in the drug literature of amprenavir commonly causing skin rashes and rarely Stevens–Johnson syndrome, and warnings about taking these protease inhibitors if there is a known hypersensitivity to a sulfonamide, there so far appear to be no reports of clear cases of allergic cross-reactivity with other sulfonamide drugs. Dapsone, or diamino-diphenyl-sulfone, is not, by definition, a sulfonamide, but it contains a sulfone group and the equivalent of the N^4 arylamine moiety of sulfonamide antibacterials. Dapsone is well known to cause the so-called dapsone hypersensitivity syndrome with fever, rash, lymphadenopathy, and some organ involvement (this syndrome may also be referred to as DRESS—drug reaction [or rash] with eosinophilia and systemic symptoms; see Sects. 2.2.4.5 and 3.6.3.5). Probably as a consequence of the introduction of multidrug therapy for leprosy worldwide, reports of dapsone hypersensitivity syndrome have increased dramatically in recent years. Other non-antimicrobial arylamines with the equivalent N^4 structure but without a sulfone group such as benzocaine and procainamide should also be looked at closely (Fig. 6.14). Procainamide is known to occasionally induce drug fever and some other allergic reactions including anaphylaxis and reports of anaphylaxis to benzocaine are readily found in the literature. There appears to be few, if any, reports of hypersensitivity responses to the β-blocker acebutolol. Hence, with all of these other sulfonamides and arylamines without a sulfone group, no available data indicate that adverse reactions to the drugs are linked to their structure or that they cross-react allergenically with sulfonamide antimicrobials. Nevertheless, it is certain that reactions to the antiretrovirals, dapsone, and both local anesthetics have not been investigated with the aim of identifying the precise structures provoking hypersensitivity reactions nor have investigations of possible allergenic cross-reactivity with sulfonamide antimicrobials been pursued at the structural level. All of this means that while the risk of reactions to sulfonylarylamines like fosamprenavir, to dapsone, and to arylamines like benzocaine appears small, prescribing these drugs for patients allergic to sulfonamide antimicrobials should not be avoided. Nevertheless, it



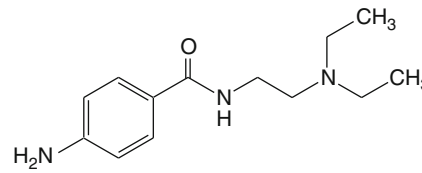
Fosamprenavir



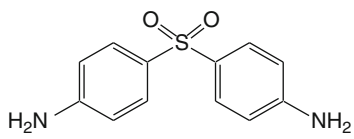
Benzocaine



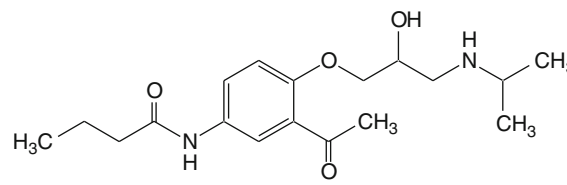
Amprenavir



Procainamide



Dapsone



Acebutolol

Fig. 6.14 The sulfonylarylamine antiretrovirals amprenavir and fosamprenavir both contain a 4-aminobenzenesulfona mido group. Both drugs and dapsone, diamino-diphenyl-sulfone, used in the management of HIV-infected patients lack the required aromatic heterocyclic ring structure attached at the N^1 nitrogen necessary for allergenic cross-reactivity with sulfamethoxazole-reactive IgE antibodies (see Fig. 6.10). Arylamines such as benzocaine, procainamide, and acebutolol which lack a sulfonyl group also fail to react with anti-sulfamethoxazole IgE antibodies

should be remembered that drug allergy is replete with examples of interesting reactions and specificities detected in the rare individual and the clinician should be aware of this and remain watchful.

6.2.2 Trimethoprim

6.2.2.1 Trimethoprim and Hypersensitivities

The subject of trimethoprim-induced hypersensitivities is not straightforward in that the literature leaves one with the impression that the real situation may not be in full view. From the authors' personal experiences of laboratory testing and investigations over many years, one might expect trimethoprim drug allergy to be far more intensively studied and worked out than it is. This conclusion is supported by patient responses, written and verbal, to questions about apparent trimethoprim "allergy," by numerous reports of adverse reactions to co-trimoxazole over a long period and the early demonstrations of true, IgE-antibody-mediated reactions to the drug. There seems to be a major contributing factor to this state of affairs. Sulfonamide antimicrobials, particularly sulfamethoxazole, have been the subject of interest and study by many clinical and laboratory-based research groups since

sulfonamides were introduced into medicine and especially since co-trimoxazole, the combination of sulfamethoxazole and trimethoprim, was introduced (see sulfonamide antimicrobials, this chapter). Adverse reactions to the sulfonamide quickly became apparent and interest in these reactions was spurred by the drug combination's high incidence of adverse reactions in immunocompromised HIV-infected patients where it had become recognized as a highly valuable treatment in combating *P. jirovecii* infections. Although there is little doubt that many co-trimoxazole-induced adverse reactions are provoked by the sulfamethoxazole component, perusal of many studies shows that it is often assumed to be the culprit drug without thorough investigation of the possible contribution of the trimethoprim component. It is not unusual to read papers on hypersensitivity reactions to co-trimoxazole where all clinical or laboratory investigations are directed at sulfamethoxazole and trimethoprim is not even mentioned let alone investigated.

Whether trimethoprim is relatively free of delayed hypersensitivity effects or whether its apparently less troubling adverse effects are due to investigative neglect, it does appear that the drug has not greatly worried allergists and dermatologists. Apart from rare reports of hypersensitivity pneumonitis, some adverse skin reactions including erythematous papular skin eruption, and toxidermias including toxic epidermal necrolysis, almost all reports of trimethoprim-induced hypersensitivities are of the immediate kind. In 936 reports of drugs frequently associated with anaphylaxis (that is, when a causal relationship was judged to be certain or probable) in The Netherlands between 1974 and 1994, sulfamethoxazole with trimethoprim was implicated in 12 and trimethoprim alone in 11 cases. In addition, a number of individual case reports of anaphylaxis to trimethoprim, some with and some without accompanying investigations for evidence of IgE antibodies, have been published. A 1996 study of eight patients who experienced anaphylaxis to co-trimoxazole (discussed below) revealed seven patients with IgE antibodies to trimethoprim and one with IgE to both trimethoprim and sulfamethoxazole. It was therefore surprising to see a published study as late as 1998 with the stated aim of determining whether the trimethoprim component of co-trimoxazole can cause an anaphylactic reaction and the conclusion that anaphylaxis to the drug combination is not always caused by sulfamethoxazole was equally surprising since it had already been clearly established. This was again, a further example of a mind-set that prejudged the inherent allergenicity of sulfamethoxazole to the exclusion of a drug already well known to occasionally provoke severe type I allergic responses.

6.2.2.2 Type I IgE Antibody-Mediated Reactions to Trimethoprim

Studies have been few but the diagnostic procedures applied so far for trimethoprim and sulfamethoxazole, and the immunochemical definition of the drug allergenic determinants, have provided a firm basis for the clinician to confidently diagnose and distinguish immediate allergic reactions to these two drugs.

Skin testing details for trimethoprim are hard to find and no validation studies for skin testing with this drug appear to have been done, but for prick tests on patients with suspected allergy to co-trimoxazole, Septrin Parenteral Infusion® (Wellcome) containing 16 mg/ml of trimethoprim (0.055 M) and 80 mg/ml of sulfamethoxazole is useful in an initial examination. For intradermal testing, trimethoprim is dissolved in the minimum quantity of 0.1 M sodium hydroxide before diluting with physiological saline to dilutions of 0.01, 0.1, and 1 mg/ml (0.034 M) and injecting 0.02 ml quantities. Physiological saline containing the same amount of sodium hydroxide is used as a control solution. There are other reports of successfully employing trimethoprim at a concentration of 20 mg/ml, that is, 0.069 M, for skin prick testing.

IgE antibodies to trimethoprim were first demonstrated in the mid-1980s. Immunochemical investigations employing trimethoprim covalently coupled via a spacer arm to Sepharose in quantitative hapten inhibition experiments with carefully selected analogs provided insights into the precise structures of the drug recognized by

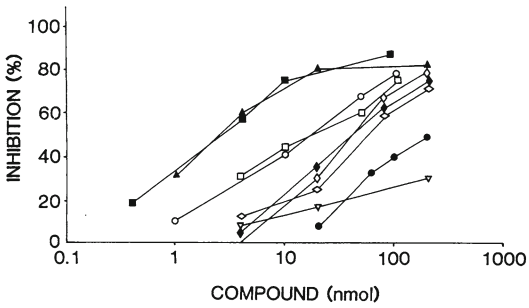
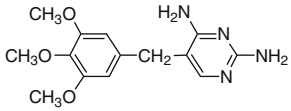
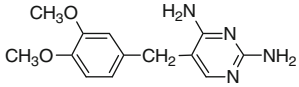
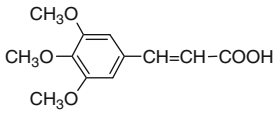
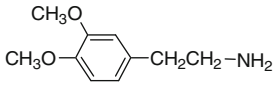
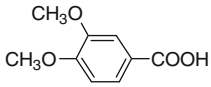
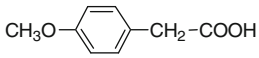
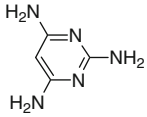
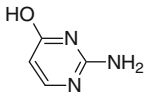
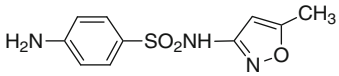


Fig. 6.15 Specific inhibition by trimethoprim and some structurally related compounds of the reaction of IgE antibodies from a trimethoprim-allergic patient with the drug. The IgE antibodies showed specificity for the 3,4-dimethoxybenzyl group of trimethoprim. Key to symbols: (*open circle*) trimethoprim; (*filled circle*) 6-hydroxytrimethoprim; (*open square*) 6-chlorotrimethoprim; (*filled square*) diaveridine; (*filled triangle*) 3,4-dimethoxyphenylethylamine; (*vertical open diamond*) 3-(3′,4′,5′-trimethoxyphenyl)-propionic acid; (*filled diamond*) 3,4-dimethoxybenzoic acid; (*inverted triangle*) 4-methoxyphenylethylamine; (*horizontal open diamond*) 3,4,5-trimethoxycinnamic acid. See also Table 6.3 and Fig. 6.16 (reproduced with permission from Smal MA et al. Allergy 1988; 43: 184)

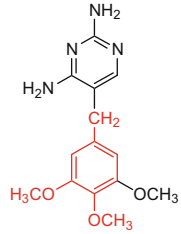
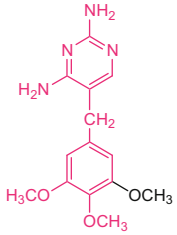
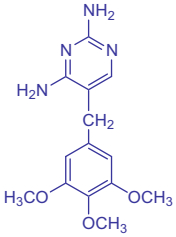
complementary IgE antibodies in the sera of trimethoprim-allergic patients. Initial examinations with sera from two allergic patients showed the clear presence of trimethoprim-binding IgE antibodies and inhibition patterns of antibody binding indicated specificity of binding and the probable existence of more than one allergenic determinant on the trimethoprim molecule. In the example shown (Fig. 6.15), the most potent inhibitors (that is, the structures showing the best fit for the IgE combining sites) were diaveridine and 3,4-dimethoxyphenylethylamine and these and the other inhibition results indicated that the trimethoprim determinant recognized was the 3,4-dimethoxybenzyl group which is almost identical to one-half of the trimethoprim molecule. Reinforcing this conclusion was the almost complete absence of inhibition seen with structures representing the other half of the trimethoprim molecule, viz., 2-amino-4-chloro-6-methylpyrimidine, 2-amino-4-hydroxy-6-methylpyrimidine, and 4-amino-5-aminomethyl-2-methylpyrimidine. More detailed follow-up investigations with an extensive range of analogs of the trimethoxybenzyl and diaminopyrimidine rings of trimethoprim identified two structures complementary to the IgE antibody combining sites, again the 3,4-dimethoxybenzyl group but also the combined trimethoxybenzyl and diaminopyrimidine rings of trimethoprim. In an attempt to more precisely define the fine structural specificity differences of trimethoprim allergenic determinants, further quantitative hapten inhibition studies were undertaken with sera from eight patients, all females, who presented after immediate allergic reactions to co-trimoxazole. Immunoassays with trimethoprim and sulfamethoxazole-solid phases revealed that all the patients had IgE antibodies to trimethoprim while one also showed a weak IgE antibody response to the sulfonamide. Three distinct patterns of inhibition were seen (Table 6.3). In group 1, comprising sera I1, I2, A1, A2, and I3, diaveridine, which differs structurally from trimethoprim by the absence of a single methoxy group, was essentially equally as potent an inhibitor as the "parent" compound. Other compounds representing the trimethoxybenzyl end of the trimethoprim molecule such as 3,4,5-trimethoxycinnamic acid and 3,4-dimethoxyphenylethylamine were also significant inhibitors while structures with only one methoxy group attached to the ring, e.g., 4-methoxyphenylacetic acid, and structures representing the other end of the trimethoprim molecule, e.g., 2,4,6-triaminopyrimidine and 2-amino-4-hydroxypyrimidine, were essentially inactive. With serum I4, once again trimethoprim and diaveridine were equally well recognized, but other compounds lacking one end or the other of the trimethoprim structure were either very weak or non-inhibitors. Group 3 showed the simplest profile with only trimethoprim showing inhibitory activity; significantly, diaveridine was without activity. Sulfamethoxazole showed no significant inhibition with six of the sera, but the small amount of inhibition seen with sera I4 and I5 might indicate weak cross-reactivity with the pyrimidine ring of trimethoprim (Table 6.3). Taken together, these data demonstrate the existence of at least three different trimethoprim allergenic determinant structures complementary to the combining sites of trimethoprim-reactive

Table 6.3 Inhibition of trimethoprim–IgE antibody interactions in the sera of trimethoprim-allergic patients by trimethoprim and some structurally-related compounds

	% Inhibition of IgE antibody binding to TMP–Sepharose with 100 nmol of compound in sera							
	Group 1					Group 2	Group 3	
Compound and structure	I1	I2	A1	A2	I3	I4	I5	A3
Trimethoprim 	**94**	**96**	**92**	**84**	**91**	**94**	**72**	**49**
Diaveridine 	**76**	**90**	**97**	**85**	**92**	**86**	3	0
3,4,5-Trimethoxycinnamic acid 	**87**	**60**	**84**	**40**	**50**	14	10	0
3,4-Dimethoxyphenylethylamine 	**63**	**76**	**93**	**60**	**80**	24	11	0
3,4-Dimethoxybenzoic acid 	**35**	**41**	**68**	0	26	9	20	0
4-Methoxyphenylacetic acid 	3	0	5	0	0	17	18	0
2,4,6-Triaminopyrimidine 	2	10	0	3	10	10	25	0
2-Amino-4-hydroxypyrimidine 	9	0	0	2	0	0	13	0
Sulfamethoxazole 	6	3	10	13	13	24	25	0

(continued)

Table 6.3 (continued)

Compound and structure	% Inhibition of IgE antibody binding to TMP–Sepharose with 100 nmol of compound in sera								
	Group 1					Group 2		Group 3	
	I1	I2	A1	A2	I3	I4		I5	A3
Allergenic determinants identified	 3,4-Dimethoxybenzyl group					 2,4-Diamino-5-(3′,4′-dimethoxybenzyl) pyrimidine group		 Entire trimethoprim molecule	

Figures highlighted in bold indicate inhibition percentages considered to be clear, unequivocal, and significant in interpreting antibody combining site structure–activity relationships. See also Fig. 6.16)
Reproduced from Pham NH et al. Clin Exp Allergy 1996;26:1155 with permission

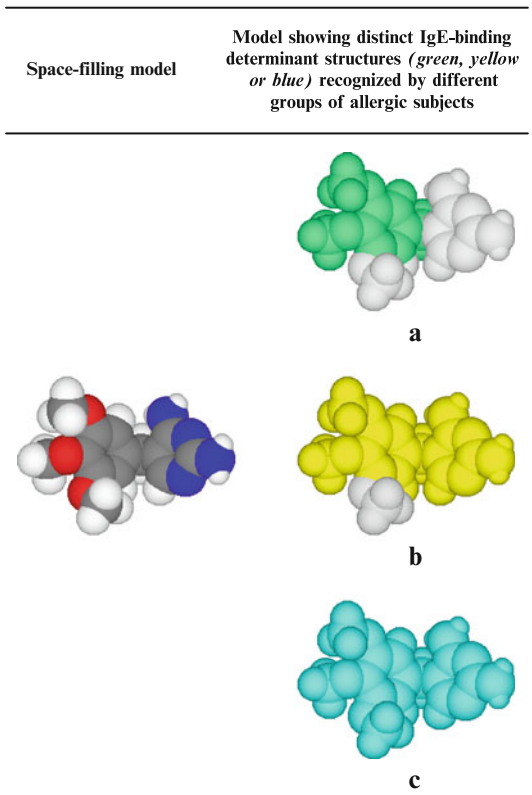


Fig. 6.16 CPK space-filling models of trimethoprim with the three IgE antibody-binding determinants shown in *green*, *yellow*, and *blue*. (**a**) The 3,4-dimethoxybenzyl determinant shown in green; (**b**) 2,4-diamino-5-(3′,4′-dimethoxybenzyl)pyrimidine determinant shown in *yellow*; (**c**) the whole trimethoprim molecule shown in blue comprises the allergenic determinant for some anti-trimethoprim IgE antibodies. See also Table 6.3

IgE antibodies in the sera of patients allergic to the drug (Fig. 6.16). One of the determinants, the 3,4-dimethoxybenzyl structure represents one-half of the trimethoprim molecule while the other two comprise the entire, or almost the entire, molecule. Of the latter two determinants, 2,4-diamino-5-(3′,4′-dimethoxybenzyl)pyrimidine differs from the third determinant, the entire trimethoprim structure, by a single methoxy group demonstrating the importance a single, small structural feature can have in antibody–drug recognition. Drug allergenic determinants, like antigenic determinants on a wide variety of peptide and non-peptide structures, show structural heterogeneity and it is likely that the IgE antibody response to trimethoprim in individual patients is also heterogeneous. While sera I5 and A3 appeared to contain antibodies of only one specificity, serum I4 may also have contained these antibodies in addition to the identified population and sera in group 1 probably contained different sized populations of antibodies of all three specificities.

6.2.2.3 T Cell Studies

These studies are in their early days. An attempt to look at trimethoprim hypersensitivity by investigating drug metabolism, processing in antigen presentation, and cross-reactivity patterns has recently been reported. It seems that antigen presentation can be dependent on or

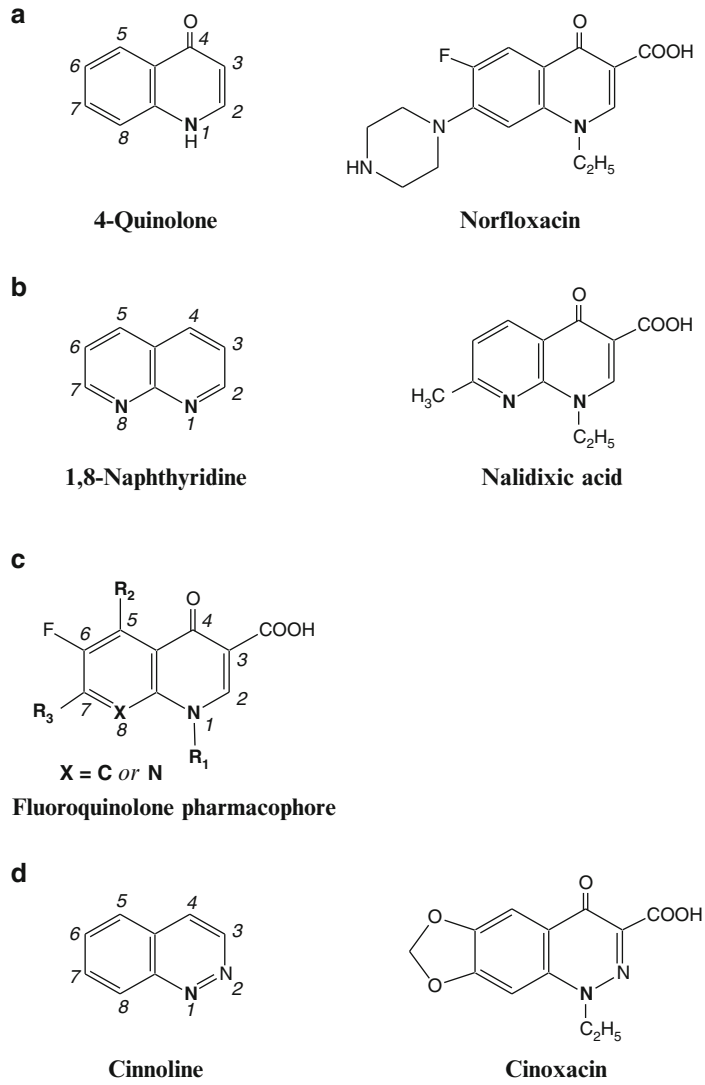


Fig. 6.17 Structures of some quinolones, a broad family of synthetic chemotherapeutic antibacterials based on the 4-quinolone (**a**) and 1,8-naphthyridine (**b**) structures. Norfloxacin and nalidixic acid, respectively, are examples of a drug from each of these groups. Cinoxacin is an example of a quinolone antibacterial based on cinnoline, an aromatic heterocyclic with two attached six-membered rings containing adjacent nitrogens at positions one and two (**d**). The basic fluoroquinolone pharmacophore is shown in (**c**). The addition of a fluorine atom at position six of the two-ring nucleus produced a 100-fold increase in the antibacterial minimum inhibitory concentration

independent of processing. Cross-reactivity studies showed that trimethoprim clones were stimulated by the close analog diaveridine and by pyrimethamine but not by other closely related structures. Presumably attempts will be made to work out a possible immune mechanism(s) for trimethoprim hypersensitivity based on the involvement of metabolites and hapten–protein complexes as was done for sulfamethoxazole.

6.2.3 Quinolones

6.2.3.1 Structures

Quinolones is the name given to a broad family of synthetic chemotherapeutic antibacterials chemically based on the 4-quinolone and 1,8-naphthyridine structures. The structures of these two quinolone nuclei and an example of a drug from each of these groups are shown in Fig. 6.17a, b. Cinoxacin is an example of a

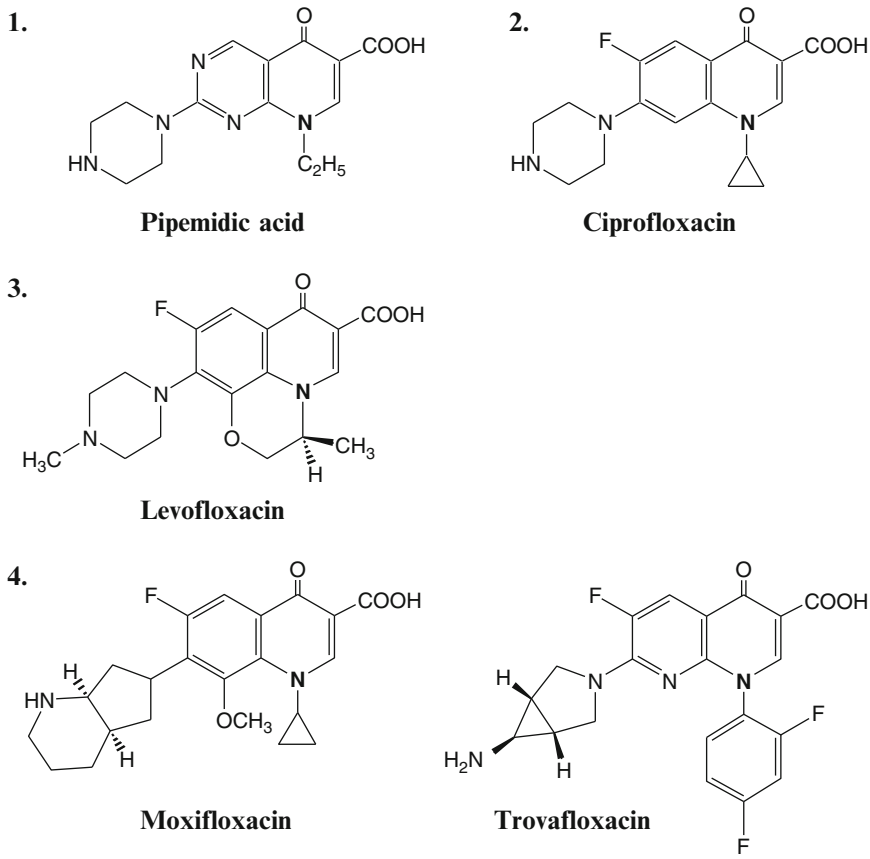


Fig. 6.18 Examples of some quinolone/naphthyridine antibacterials illustrating the structural developments over the four generations (1–4) of the drugs

quinolone antibacterial based on cinnoline, an aromatic heterocyclic with two attached six-membered rings containing adjacent nitrogens at positions one and two (Fig. 6.17d). The addition of a fluorine atom at position six of the two-ring nucleus produced a 100-fold increase in the antibacterial minimum inhibitory concentration of the resultant fluoroquinolones. The basic fluoroquinolone pharmacophore is shown in Fig. 6.17c. Nalidixic acid, generally regarded as the first of the quinolone antibacterials, was used for urinary tract infections. It was quickly followed by other so-called first generation and then by second-, third-, and fourth-generation drugs as efforts were made to increase low tissue concentrations; increase short half-lives and decrease the need for frequent dosage; broaden the spectrum against *Pseudomonas aeruginosa* and gram-positive organisms such as the staphylococci and streptococci; and produce agents with adequate pharmacodynamic profiles. These changes were effected by chemical incorporation of judiciously selected groups such as the cyclopropyl substituent used to replace the *N*-1 ethyl of norfloxacin to gain the increased bioavailability seen with ciprofloxacin. Another example is the addition of a piperazine ring to the C-7 position (Fig. 6.18) to increase activity against gram-negative organisms. The range of quinolone and naphthyridines now available in oral and parenteral forms provide far greater bioavailability and work against a much broader range of organisms, including many difficult anaerobes, than the earlier generation drugs. Examples of some quinolone/naphthyridine antibacterials illustrating the structural developments over the four generations of drugs are shown in Fig. 6.18.

6.2.3.2 Quinolones and Hypersensitivities

Chemists, pharmacologists, and toxicologists are always looking for likely structural correlates between chemical groupings and adverse reactions to a drug. Some examples of this with the quinolones include speculations concerning trovafloxacin and hepatic eosinophilia, eosinophilia of the lungs and other immunological effects caused by tosufloxacin, and the suspected involvement of temafloxacin in a hemolytic uremic-like syndrome. Metabolites that share structural features not yet identified on some quinolones especially the naphthyridones, are suspected of being the agents responsible for some immunologically mediated reactions to these drugs. Many reports state that reactions to quinolone antibacterials are rare, but the literature does not always reflect this. An incidence of adverse reactions of 2–10 % has been described as "fairly safe." Included in the list of reactions are gastrointestinal complaints, central nervous system symptoms, anaphylactoid reactions, and skin conditions such as maculopapular and urticarial skin rashes, dermatitis, and vasculitis. A frequency of 1 in 50,000 treatments has been reported for quinolone-induced immune-mediated hypersensitivities while a recent study of T cell-mediated reactions to quinolones stated a combined incidence of 2–3 % for immediate and delayed hypersensitivities. One recent large survey from Thailand covering a 4-year period looked at 166,736 patients treated with fluoroquinolones and found prevalences of adverse and cutaneous adverse reactions of 0.13 and 0.09 %, respectively. Prevalences of between 0.04 and 0.37 % were seen for cutaneous reactions to individual fluoroquinolones although the full range of quinolone drugs to which the population had been exposed over the period and prior to it was not provided. Skin reactions have been reported with incidences of up to 2.5 % with maculopapular exanthema and fixed drug eruptions being the most common manifestations. Rare cases of Stevens–Johnson syndrome and toxic epidermal necrolysis have occurred.

6.2.3.3 Immediate Hypersensitivity to Quinolones

Immediate hypersensitivity reactions that may involve skin rashes, pruritus, respiratory distress, and shock are the most frequently reported immune-mediated reactions with a frequency of from 0.4 to 2 %. Descriptions of anaphylactic reactions to a number of different quinolone drugs, especially ciprofloxacin but also including nalidixic acid, pipemidic acid, pefloxacin, ofloxacin, norfloxacin, levofloxacin, and moxifloxacin, are not hard to find. As with many other drugs, reactions can occur on first exposure so sensitization by previously taking a quinolone antibacterial does not seem to be required. Ciprofloxacin has been the most commonly used quinolone and the drug responsible for most of the anaphylactic/anaphylactoid reactions. A literature search for the period 1960–2009 identified 64 cases of anaphylactoid reactions considered to be probably related to ciprofloxacin. Although the manufacturer of ciprofloxacin lists pulmonary edema as an adverse event associated with the drug, no reports of this reaction were identified in the survey.

6.2.3.4 Skin Tests

For the diagnosis of immediate allergic reactions to quinolones, skin tests including prick, intradermal, and patch tests have been employed. Skin tests with these drugs have been described by a number of investigators as not useful for diagnosis, providing little information, and not suitable for determining specific tolerances to individual drugs. Authors from a few other studies, however, have concluded that quinolone skin tests are a useful tool for the study of hypersensitivity to quinolones. A highly suggestive history of quinolone allergy was found to be associated with positive skin tests and skin tests were therefore claimed to be helpful in predicting the results of challenge tests. The following quinolone drug concentrations have been used for prick testing in subjects with suspected quinolone allergy: Ciprofloxacin 0.02 mg/ml; levofloxacin 5 mg/ml; pipemidic acid, ofloxacin, norfloxacin, levofloxacin, moxifloxacin 400 mg tablet ground up and

suspended in physiological saline; trovafloxacin 200 mg tablet in saline. For intradermal tests: Ciprofloxacin 0.005, 0.02, and 0.05 mg/ml; levofloxacin 0.005 and 0.05 mg/ml; moxifloxacin 0.005 and 0.05 mg/ml. All the drugs were initially used in prick tests and then injected (0.05 ml) intradermally if the prick test result proved negative. A recent study recommended a nonirritant intradermal test concentration of 0.0067 mg/ml for ciprofloxacin. Skin testing with quinolones has revealed a curious finding that so far seems to have been neglected or ignored. At least five separate studies have found positive skin test results to quinolone antibacterials in healthy control subjects. This observation is highly interesting and will be returned to below in the discussion on IgE antibodies to quinolones. Patch testing does not seem to have been widely used. In 101 patients with a history of hypersensitivity (immediate or delayed) to quinolones in temporal relation to administration of fluoroquinolones, 71 were excluded by tolerated oral challenge tests and patch testing was consistently negative. Six patients developed anaphylaxis, and interestingly, three of these were skin prick test positive and three were skin prick test negative to fluoroquinolones.

In summary, skin testing to diagnose immediate allergies to quinolones is currently not widely and confidently accepted and practiced although some investigators advocate its use, and even in cases where correlations with challenge tests are not perfect, results with some patients show good correlation.

6.2.3.5 Challenge Tests

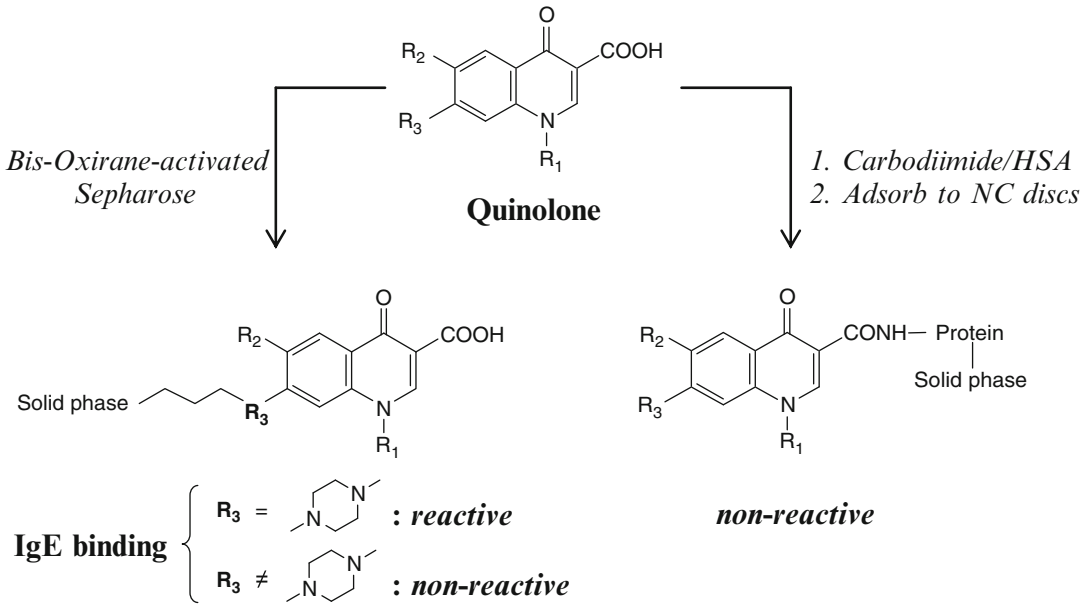
See Sect. 4.4 for a detailed discussion of drug challenge tests.

A number of investigators advocate the need to carry out challenge tests to confirm allergic sensitivity or tolerability to quinolones. Some negative skin test results with positive oral challenge results have indicated the advisability of performing oral challenge tests before selecting quinolone as safe to administer or as a safe alternative drug. However, quinolone skin tests have still been claimed to be useful for the study of type I allergic responses since they help in deciding whether or not a challenge test should be employed. In one retrospective analysis, a negative skin test preceded a negative challenge test in 94 % of challenged patients and only 5 % of patients had a negative skin test and a positive challenge test. On the other hand, only 50 % of patients with a positive skin test had a positive challenge test. In a typical challenge test, increasing doses of the drug are ingested or injected every 30 min until the therapeutic dose is reached or until symptoms of a reaction occur. The patient is kept in the clinic under observation for 45 min after the last dose. A test is considered positive if any signs or symptoms of the patient's previous reaction occur within 24 h of the last challenge dose. The result is scored as negative if no sign of drug hypersensitivity occurs after the usual therapeutic dose is administered. Doses of some quinolone drugs administered in a typical study are ciprofloxacin and levofloxacin 50, 125, 250, and 500 mg (one tablet); moxifloxacin 40, 100, 200, and 400 mg (one tablet). Blood pressure and heart rate are monitored after each dose, resuscitation equipment and drugs are available, and challenges should not be undertaken without the patient's written informed consent.

There appears to be no delayed reactions to quinolone antibacterial drugs following challenge tests or skin tests.

6.2.3.6 IgE Antibodies to Quinolones

Early attempts to demonstrate specific IgE antibodies to quinolones appear to have been unsuccessful due to difficulties associated with binding the drugs to a suitable solid phase carrier. The first successful demonstration of IgE antibodies apparently specific for quinolone antibacterials employed bis-oxirane coupling of drug to the solid phase Sepharose and was carried out in 1995–1996 in the authors' laboratory in Sydney employing sera from Italian patients supplied by Professor R. Zerboni of Florence. Unlike Europe, quinolones are not extensively prescribed in Australia and cases of hypersensitivity are consequently rare. Experiments were initially directed at determining the optimum position(s) for coupling on the quinolone or naphthyridine nucleus and the best chemical procedure to



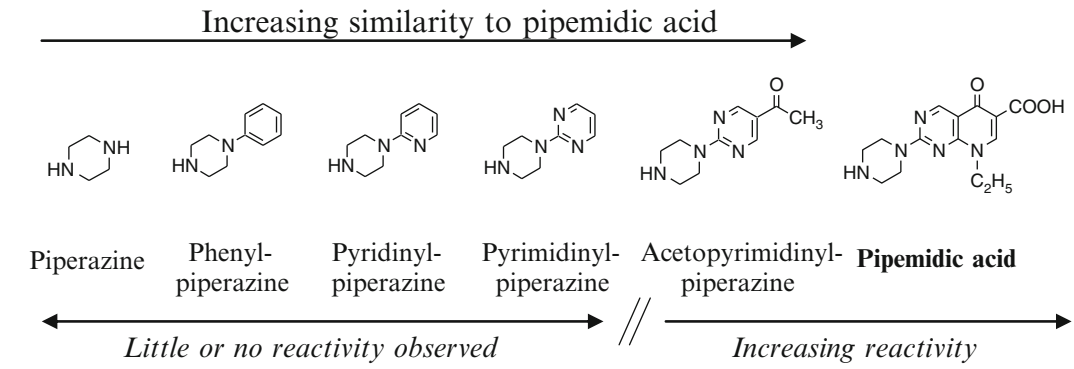


Fig. 6.19 Preparation of quinolone-solid phase covalent conjugates. Reactions were selected to attach an insoluble carrier to each side of the two-ring nucleus as part of a strategy directed at determining the optimum position(s) for coupling on the quinolone or naphthyridine nucleus and ultimately achieving maximum binding to drug-reactive IgE antibodies. To further evaluate the binding and importance of the substituted 4-quinolone ring, compounds representing increasing portions of the pipemidic acid molecule were selected, coupled to a solid support, and examined for IgE antibody binding with patients' sera. Note that the names of these latter compounds have been simplified for ease of inclusion in the figure (compare text) (adapted and reproduced with permission from Baldo BA Curr Opin Allergy Clin Immunol 2001; 1: 327)

achieve maximum binding to drug-reactive IgE antibodies. Reactions were selected to attach an insoluble carrier to each side of the two-ring nucleus. With pipemidic acid for example, carbodiimide activation of the 3-carboxyl group and coupling to human serum albumin before adsorption of the drug–albumin complex to nitrocellulose discs were effected by reactions on only

one side of the molecule. For linkage of carrier to the other side of the molecule, base-catalyzed nucleophilic addition of the nitrogen of the piperazine ring attached at position seven of the quinolone nucleus, to the epoxide of bis-oxirane-activated Sepharose, was utilized. Both strategies for linkage are represented in Fig. 6.19. Experiments using the drug-solid phases prepared by coupling functional groups on each side of the molecule showed unequivocally that conjugates prepared by coupling through the 3-linked carboxyl group were not reactive with IgE antibodies in allergic patients' sera while conjugates prepared by linkage to the opposite side of the molecule were clearly reactive. This indicated that the allergenic determinant structure complementary to the IgE antibody combining sites comprised part of, or perhaps, the entire 3-carboxy-4-quinoline ring. Total absence of antibody binding after coupling to the 3-carboxyl group suggests that it is probably an important feature of the allergenic site. To further evaluate the binding and importance of the substituted 4-quinolone ring, compounds representing increasing portions of the pipemidic acid molecule (Fig. 6.18) were selected, coupled to bis-oxirane-activated Sepharose and examined for IgE antibody binding with patients' sera. Little or no reactivity was found with piperazine and the next three simplest derivatives of piperazine, 1-phenylpiperazine, 1-(2-pyridyl)piperazine, and 1-(2-pyrimidinyl)piperazine (Fig. 6.19), although one serum from a patient allergic to quinolones reacted with 1-(2-pyridyl)piperazine. However, with the structure of the selected compounds that showed the closest similarity to the parent drug, 1-(5-aceto-(2-pyrimidinyl))piperazine, clear IgE antibody-binding was obtained although antibody binding was weaker than the binding seen with pipemidic acid. Although all of these findings pointed to the development of a successful assay for the detection of quinolone-reactive IgE antibodies and inhibition studies with free quinolone drugs demonstrated specificity to quinolones and cross-reactivities between different quinolones, reactivity of the Sepharose conjugate with occasional sera from a normal, nonallergic, healthy control subjects was unexpected and difficult to interpret. In addition, "normal" sera that reacted with the drug conjugate were inhibited by free pipemidic acid and some other quinolones, suggesting that the reactions seen were quinolone specific. These findings seemed to confirm that quinolone-reactive IgE antibodies occur not only in sera from quinolone-allergic patients but also in some apparently "normal" controls, i.e., sera from individuals with no known or apparent allergic sensitivity to quinolone antibacterial drugs. The origin of these antibodies remains obscure but it should be remembered that immediate allergic reactions to quinolones, and to a number of other drugs (with the best example being neuromuscular blocking drugs), often occur on first exposure in subjects with no previously suspected allergy to the drug. It would be interesting to determine the frequency of occurrence of such subjects and sera in the normal and drug-allergic populations and speculate on the likely possible source of sensitization. This curious finding also adds interest to the results showing positive skin tests in apparently normal, healthy controls, results obtained in a number of careful investigations. Sera from these subjects too should be tested for IgE antibody binding to quinolones. "Nonspecific" histamine release by direct action of quinolones on mast cells has been offered by some as an explanation for the "false" positive skin test results. This may indeed be the explanation but in one skin test study with quinolones where numbers were given, 5 of 37 subjects who were classed as not hypersensitive to quinolones on the basis of negative challenge tests had positive skin tests. If quinolone-induced histamine release were involved one might expect all, or perhaps even most, of the subjects to show skin reactions.

Some radioimmunoassay results obtained with bis-oxirane-linked quinolones using the strategy and chemical procedures described above, were published from Europe in 2004 and another drug–Sepharose radioimmunoassay for detection of IgE antibodies to quinolones has recently appeared. In the former study, 30 of 55 patients (54.5 %) who showed an immediate

reaction to a quinolone drug yielded positive results when tested in the immunoassay 1–48 months after the reaction. The interval between blood sampling and the reaction to the drug may be important since radioactive uptakes were significantly higher in subjects sampled within 8 months of the adverse reaction. In the more recent Sepharose-bis-oxirane-based radioimmunoassay tests for ciprofloxacin, levofloxacin, and moxifloxacin were undertaken and shown to be positive for quinolone-reactive IgE antibodies in 12 of 38 patients with severe immediate allergic reactions to quinolones. With the same subjects, 27 patients (71 %) were positive in the basophil activation test when ciprofloxacin, levofloxacin, and moxifloxacin were analyzed together. The high sensitivity of the basophil activation test in this study indicates that the test may be a valuable adjunct for drug provocation in the diagnosis of immediate allergic reactions to quinolones, but results with the test in some other hands have not been as encouraging. In three separate investigations, the test was positive in none of 12 patients who had immediate reactions after oral administration of a quinolone, in none of four patients with symptoms of anaphylaxis after an oral challenge with fluoroquinolones, and in 17 of 34 patients who experienced an immediate hypersensitivity reaction within 1 h of quinolone administration. In the first of these investigations, the authors concluded that the basophil activation test along with skin testing was not helpful in establishing a diagnosis or in predicting cross-reactivity to quinolones. A very recent BAT study on 34 patients with immediate hypersensitivity to quinolones found 17 positive and 17 negative to the suspected quinolone. Fifteen of the negative group tolerated the reintroduction of the drug (two were skin test positive) causing the investigators to state that a negative BAT is valuable information to consider when deciding whether or not to perform challenge tests on patients with a history of an immediate reaction to quinolones. More studies are needed with the basophil activation test before its diagnostic application to quinolone immediate hypersensitivity is accepted and validated or further questioned.

6.2.3.7 Cross-reactions of Quinolones

There is no doubt that immunologic cross-reactivity between quinolone antibacterials exist; the questions are how extensive is it and what is the clinical relevance? Views range from the need to avoid any quinolone if a patient is allergic to one, to the belief that some patients show good tolerance to quinolones selected by drug challenge tests. For example, levofloxacin was found to be a tolerated alternative for four out of five patients allergic to ciprofloxacin and for two patients who reacted to norfloxacin. A high degree of cross-reactivity between the fluoroquinolones has been reported and cross-reactivity between first- and second-generation quinolones seems to be generally accepted. Overall though, there seems to be no reliable and confident way of predicting cross-reactivity short of employing series of laborious and potentially dangerous challenges and this means that cross-reactions need to be looked at patient by patient.

At the level of serum IgE antibodies, 9 of the 55 patients discussed above reacted to more than one quinolone and 24 of 30 patients (80 %) showed IgE antibody reactivity with more than one quinolone. The comment has been made that cross-reactivity between quinolones "seems to be related to the molecular ring common to all quinolones," and although this seems self-evident since cross-reactivity must ultimately have some common chemical basis, the chemical picture is not so simple and straightforward. As the structures set out in Fig. 6.17 show, three different ring systems are found in the so-called quinolone antibacterial drugs and, regardless of the particular nucleus, different added substituents, particularly at positions one, six, seven, and eight, lead to differences in chemical, physical, and pharmacological properties between many individual drugs. These differences will include the extent to which individual drugs are recognized by IgE antibodies induced by a particular quinolone. As outlined above and summarized in Fig. 6.19, the position of attachment of the solid phase carrier necessary for development of an immunoassay to detect IgE demonstrated the importance of the 3-carboxy-4-quinoline "face" of quinoline and naphthyridine derivative structures

in reacting with antibodies. This immediately explains the poor recognition of some quinolone-reactive IgE antibodies with cinoxacin that has an extra nitrogen at position two on that face. No doubt other IgE antibodies with different fine structural recognition spectra occur so one can predict that depending on this antibody heterogeneity, on the point of attachment to the quinolone molecule of drug-solid phases, and on the selection of analogs chosen for side-by-side inhibition studies, findings will differ, sometimes quite markedly, depending on the quinolones examined for cross-reactivity. Once again, the most informative and efficient way to clarify the apparently complex question of allergenic cross-reactivity of a family of drugs is to carry out carefully planned in vitro quantitative hapten inhibition studies using suitable IgE antibody immunoassays and a judiciously selected panel of quinolone drugs and other structural analogs some of which will not be therapeutic agents. For clinical relevance comparisons, the results of such examinations should be looked at alongside results of complementary skin test and perhaps basophil activation tests. It seems that the picture of IgE antibody-mediated allergic recognition of the quinolone drugs is still substantially incomplete.

6.2.3.8 Delayed Reactions to Quinolones

Non-immediate reactions to quinolones occur but they are not encountered as often as immediate reactions and in-depth studies are so far few. Some of the more often-seen delayed reactions are fixed drug eruptions and maculopapular exanthemas where a T cell mechanism has been demonstrated. Specific T cell clones were identified from patients with ciprofloxacin-induced maculopapular exanthems and about half of the clones proved cross-reactive with related drugs. Reexposure studies in patients with exanthems revealed that cross-reactivity is in fact lower than this. Cellular tests such as lymphocyte transformation tests were judged to be not very useful. However, the lymphocyte transformation test was said to have confirmed the involvement of T cells when peripheral blood mononuclear cells proliferated in vitro in response to ciprofloxacin, norfloxacin, or moxifloxacin in six patients with delayed hypersensitivity to the drugs. Patch tests were positive after 24 and 48 h in three of the patients. Ciprofloxacin- and moxifloxacin-specific T cell clones were generated and used with different quinolone drugs to examine cross-reactions. Three patterns of cross-reactivity were observed: clones that reacted only with the eliciting drug and clones with limited or broad cross-reactivity. T cell clones were said to be recognized directly without processing within the immune system.

6.2.4 Chlorhexidine

Chlorhexidine is a synthetic cationic bis-biguanide that can be viewed as two biguanide groups each linked to a terminal 4-chlorophenyl group with the resultant chlorguanide structures connected by a hexamethylene bridge (Fig. 6.20). Since its introduction in 1954 as an antiseptic and disinfectant, chlorhexidine, usually as the gluconate or acetate salt, has found widespread application in a myriad of products used domestically, industrially, and in the medical environment. Some everyday products that include chlorhexidine are toothpastes, gargles, antiseptic creams, liquid disinfectants, mouthwashes, contact lens fluid, contraceptives, lubricants, and cosmetics. Medically, as an antimicrobial agent, it is in liquids, gels and creams, ointments, plasters, dressings, and suppositories and is used on skin, mucous membranes, wounds, and burns and to disinfect surfaces and instruments. These lists are nowhere near complete indicating how common and extensive is human contact with chlorhexidine and as a result how easy is the opportunity for contact with the compound.

Anaphylaxis to chlorhexidine can occur, and although rare, it is these reactions that have aroused allergists' attention to the agent. Many drugs provoke an occasional anaphylactic or anaphylactoid response but with chlorhexidine there are some features of the cases that were unanticipated, surprising, and, initially at least, obscure. The first reported cases of anaphylaxis

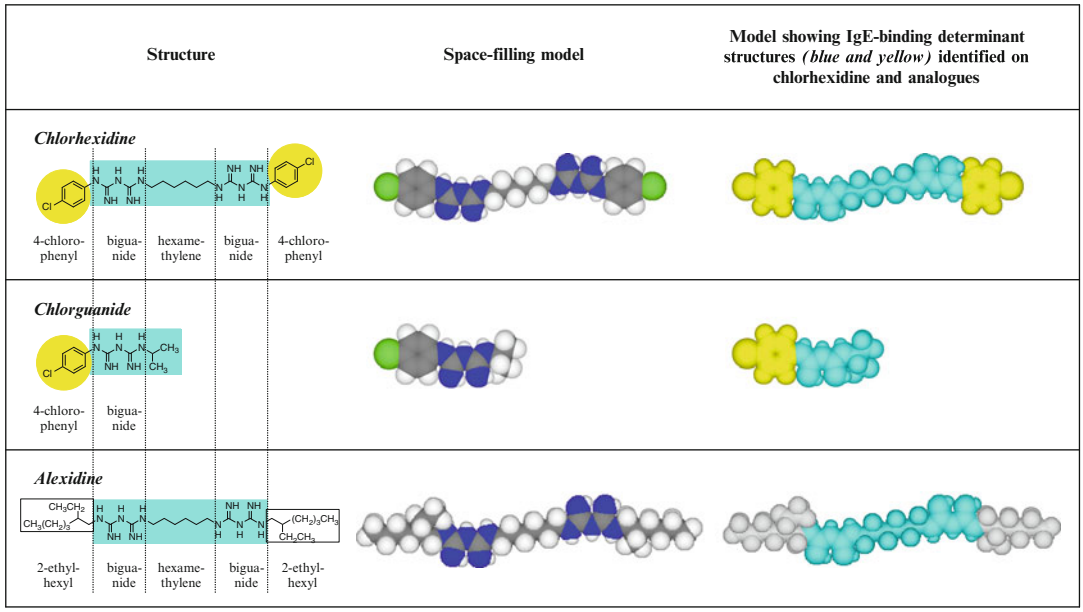


Fig. 6.20 Chlorhexidine is a synthetic cationic bis-biguanide that can be viewed as two biguanide groups each linked to a terminal 4-chlorophenyl group (*yellow*) with the resultant chlorguanide structures connected by a hexamethylene bridge (*blue*). Space-filling models showing the relative allergenic (IgE antibody-binding) activities of different regions of the chlorhexidine model. Only chlorhexidine, chlorguanide, and alexidine showed inhibitory activity of anti-chlorhexidine IgE with the parent compound being most active (*yellow* and *blue*). All of the compounds selected to represent the terminal chlorinated aromatic ring of chlorhexidine and the subterminal guanide structures were non-inhibitory. Clear antibody recognition of alexidine demonstrated that the antibody combining sites extended beyond the chlorguanide structure and included the hexamethylene central sequence. Taken together, these results showed clear antibody combining site recognition of the identical arms of the chlorhexidine molecule, but the structure most complementary to the sites was the entire chlorhexidine molecule. See also Table 6.4 (adapted and reproduced with permission from Pham NH et al. Clin Exp Allergy 2000; 30: 1001)

were in 1985 in Australia and Japan and the first description of chlorhexidine-induced anaphylaxis via the urethral route appeared in 1992. The latter paper appears to have been the forerunner of a number of reports of anaphylaxis elicited during urologic procedures when chlorhexidine was exposed to urethral and bladder mucosa. A review of the literature in 2002 revealed that 21 of 66 cases of anaphylaxis to chlorhexidine up to that time were precipitated by urethral exposure to the drug. The same review revealed that 27 patients had an immediate reaction after chlorhexidine was applied to mucous membranes and 16 patients experienced anaphylaxis after introduction of a chlorhexidine-coated central venous catheter. Among 50 cases of adverse reactions to chlorhexidine reported to the Japanese Ministry of Welfare between 1967 and 1984, nine cases were of anaphylactic shock and all 50 cases were associated with mucosal application of the drug. The agency's response was a recommendation that chlorhexidine should not be used on mucous membranes. In one of the original reports of anaphylaxis, cleansing the skin with 0.5 % chlorhexidine acetate prior to a skin graft induced the reaction. This example, the fact that chlorhexidine is not a drug administered by anesthetists, and its presence in what has been described as "covert" forms all make it easy to see why this widely distributed and used antimicrobial agent was, and presumably still is, often overlooked as a provoking source of anaphylaxis. The presence of chlorhexidine as a coating for catheters or in urethral lubricants, for example,

Instillagel® which contains 0.25 % chlorhexidine, 2 % lidocaine, and mixed hydroxybenzoates, demonstrates why its involvement in inducing allergic reactions might in some cases remain unrecognized. A survey in 2005 concluded that chlorhexidine accounted for 27 % of overlooked perioperative hypersensitivity reactions.

6.2.4.1 Delayed Hypersensitivity Reactions to Chlorhexidine

Although reactions with an immediate type I mechanism are the most important chlorhexidine-induced allergic reactions medically and receive most attention, delayed hypersensitivity reactions to the drug do occur and immediate and delayed reactions can occur in the same patient. Exposure to chlorhexidine, usually prolonged, can lead to contact sensitization and allergic contact dermatitis and stomatitis. In a study designed to examine sources of exposure and sensitization to chlorhexidine and to obtain information on the prevalence of sensitization and contact allergy, over 7,500 general dermatology patients with suspected contact allergy in Finland were patch tested with chlorhexidine digluconate 0.5 %. A positive test was seen in 0.47 % of patients with five patients showing dermatitis or stomatitis caused by topical medicaments containing chlorhexidine and the antiseptic was judged to have contributed to current dermatitis in 11 patients. Only 16 sensitized patients could recall a history of previous exposure while four appeared to have had no exposure. The conclusion was that creams, disinfectants, and so-called oral hygiene products are the main sources of contact sensitization to chlorhexidine and its presence in cosmetics may delay the improvement in eczema in some patients. Chlorhexidine skin swabs, irrigation solutions, and skin "prep" solutions used prior to surgery have been associated with contact dermatitis which was found by patch testing to occur with incidences of 2.5 % in a Danish skin clinic and 5.4 % in atopic patients. In another study of dermatology patients, the incidence of type IV hypersensitivity to chlorhexidine was determined to be between 1 and 2.5 %. In healthcare workers in Denmark, skin tests (including patch testing) to detect type IV hypersensitivity to the drug showed no positive reactions in 104 subjects. This is at marked variance with a Japanese study of healthcare workers that reported a 7 % incidence of contact dermatitis to chlorhexidine.

6.2.4.2 Immediate Hypersensitivity Reactions to Chlorhexidine

Incidences of anaphylaxis to chlorhexidine are not necessarily readily found or widespread and, in fact, there are no reports of any cases from many countries. This may reflect the local absence of chlorhexidine preparations, failure to recognize and/or investigate reactions, the different concentrations used in antiseptics in different countries, or absence of sensitivity to the agent. The Danish Anaesthesia Allergy Centre, established in 1998 to investigate patients referred from all over Denmark, found 4 men of 21 patients with positive skin tests to various substances tested positive to chlorhexidine. Three cases of anaphylaxis to chlorhexidine were reported in Finland up to 1999, 11 cases were seen in Australia in the period 1985–1994 and 9 in Japan between 1967 and 1984.

6.2.4.2.1 Skin Tests for Diagnosis

In the case of the immediate reaction to 0.5 % chlorhexidine acetate aqueous solution mentioned above, the same solution produced no reaction when tested on the patient's skin but a 1:100 dilution injected intradermally produced, within 1 min, a wheal that lasted more than 30 min. In another diagnostic investigation of chlorhexidine-induced anaphylaxis, application of chlorhexidine solution to the skin in dilutions of 1:10,000 to 1:1,000 proved negative but a 1 % solution was strongly positive. In the same study, skin prick tests using dilutions of 1:100 to 1:10,000 were negative but stimulation with chlorhexidine was high in the sulfidoleukotriene stimulation test (CAST®, Buhlmann Laboratories, Switzerland). In Korea, positive prick tests to chlorhexidine solution 5 % were obtained when tested undiluted and at 1:10 and 1:100 and intradermal tests with 1:1, 1:10, and 1:100 dilutions also gave positive results. For prick testing, investigators often utilize available stock solutions

(usually 1–5 %) either neat or a little diluted, e.g., up to about 1:10. The Danish Anaesthesia Allergy Centre employs a standardized investigation program in which the more widely used chlorhexidine digluconate is used at a concentration of 0.5 % in physiological saline for prick testing and 0.0002 % for intradermal testing. Prick tests are performed on the forearm with saline and histamine controls. Development of a wheal greater than half the diameter of the wheal of the positive histamine control together with a negative saline control is considered positive. Intradermal tests are performed on the back or forearm with a saline negative control. A positive test is the development of a wheal (with flare) equal to or greater than twice the diameter of the bleb formed from the volume of the injected solution. In practice, a positive test is a bleb with a diameter of 8 mm or greater. These concentrations used for skin testing are based on more than 800 negative controls for prick tests with 0.5 % chlorhexidine digluconate and more than 300 negative reactors to 0.0002 % chlorhexidine digluconate in intradermal tests.

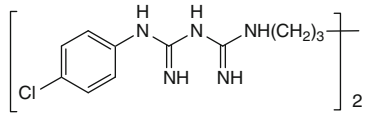
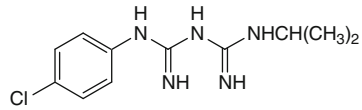
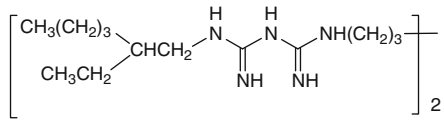
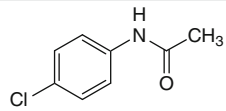
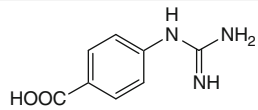
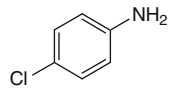
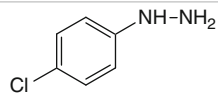
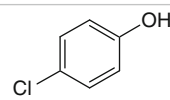
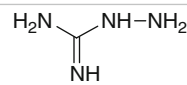
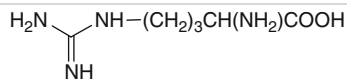
Skin prick tests on a patient who experienced an anaphylactic reaction to Instillagel® produced results that draw attention to a potential pitfall when testing for sensitivity to this preparation. The demonstration of a positive skin test to chlorhexidine after the finding of a negative skin prick test to Instillagel® was attributed to the presence of lidocaine in the gel and its effect in ablating the neurogenic wheal response required for a positive prick test.

6.2.4.2.2 IgE Antibody Tests for Diagnosis and Drug Allergen Studies

IgE antibodies were first implicated in an anaphylactic reaction to chlorhexidine in Japan in 1986. Specific skin-sensitizing IgE antibodies to chlorhexidine were demonstrated in the patients' sera by passive transfer and by an immunoassay using paper discs conjugated to chlorhexidine linked to human serum albumin. Dose-dependent inhibition of IgE antibody binding to the chlorhexidine-solid phase by both chlorguanide and the parent drug demonstrated specificity of the reaction. Attempts to identify chlorhexidine allergenic determinants were initiated nearly 20 years later when chlorhexidine linked to a bis-oxirane-activated solid phase was used in quantitative hapten inhibition studies with a serum from a patient who experienced anaphylactic episodes on three separate occasions after exposure to urethral gel containing chlorhexidine and lignocaine. Chlorguanide, which represents almost half the chlorhexidine molecule, alexidine, aminoguanidine, and arginine, which are similar to the interior structure and some substituted chlorophenyl compounds that mimic the terminal group at each end of the chlorhexidine molecule were selected for study of the chlorhexidine-reactive IgE antibody combining sites in the patient's serum. Only chlorhexidine, chlorguanide, and alexidine proved inhibitory with, expectedly, the parent compound being most active (Table 6.4). A comparison of the 50 % inhibitory concentrations of the three compounds showed that chlorhexidine was from 100 to 200 times as potent as the two analogs. All of the compounds selected to represent the terminal chlorinated aromatic ring of chlorhexidine and the subterminal guanide structures represented by aminoguanidine and arginine were non-inhibitory. Clear antibody recognition of alexidine demonstrated that the antibody combining sites extended beyond the chlorguanide structure and included the hexamethylene central sequence. Taken together, these results showed clear antibody combining site recognition of the identical arms of the chlorhexidine molecule but the structure most complementary to the sites, that is, the structure showing the "best fit," was the entire chlorhexidine molecule (Table 6.4). Figure 6.20 shows the dominant features of the fine structural recognition of chlorhexidine by the complementary IgE antibodies studied.

Many of the drug allergenic determinants identified so far comprise parts of rather than the entire molecule and complementary IgE antibodies show combining site heterogeneity sometimes identifying two or more different structures on a drug molecule (see Chap. 5, Sect. 5.1.2, Chap. 6, Sect. 6.2.2 and Chap. 7, Sects. 7.4.2.2 and 7.5.1.2 for examples). It will be interesting to see if chlorhexidine demonstrates such recognition heterogeneity. In the 1986 Japanese study, the authors concluded that

Table 6.4 Inhibition of the interaction of chlorhexidine–Sepharose complex with IgE antibodies by chlorhexidine and some structurally related compounds

Compound	Structure	% Inhibition of IgE antibody binding to chlorhexidine – Sepharose in serum at 10 nmol	100 nmol
Chlorhexidine		**81.3**	**85.9**
Chlorguanide		**34.5**	**64.0**
Alexidine		**40.5**	**53.2**
4-Chloro-acetanilide		0	0
4-Guanidino-benzoic acid		0	0
4-Chloroaniline		0	0
4-Chlorophenyl-hydrazine		0	0
4-Chlorophenol		0	0
Aminoguanidine		0	0
Arginine		0	0

Compounds with % inhibition values in bold showed significant inhibition
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chlorhexidine and chlorguanide share the same antigenic determinant. In looking at the structure of chlorhexidine from the viewpoint of binding to IgE molecules, the identical structural features at each end of the molecule raise the possibility that this inherent divalency without the need for protein binding might allow bridging of combining sites of adjacent mast cell-bound IgE molecules leading to mediator release. This prediction was made and confirmed for neuromuscular blocking agents where there are at least two allergenic determinants at distances apart of

1–1.45 nm (see Sect. 7.4.2.3). The terminal chlorophenyl groups of chlorhexidine are separated by 16 atoms of carbon and nitrogen and the IgE-reactive chlorguanide groups are bridged by a six carbon chain so the requirements of distance and flexibility needed for cross-linking at the mast cell surface may be satisfied. The chlorguanide structure has been shown to interact with IgE antibody combining sites; it remains to be seen if IgE antibodies with specificity restricted to the terminal 4-chlorophenyl group occur.

A specific test to detect serum IgE antibodies to chlorhexidine is now available in the form of an ImmunoCAP® (Thermo Scientific) test. The drug-solid phase is prepared by coupling 1-[N^5-(*p*-chlorophenyl)biguanido]-6-aminohexane which represents half of the symmetrical bivalent chlorhexidine molecule to cyanogen bromide-activated cellulose sponge.

6.2.5 Povidone–Iodine

Povidone–iodine is a chemical complex of elemental iodone, 9–12 % (w/w), with polyvinylpyrrolidone (PVP) producing an iodophor preparation that slowly releases free iodine in solution. PVP, also called polyvidone or povidone, is a water-soluble polymer of *N*-vinylpyrrolidone, chains of which can range in molecular weight from 10,000 to 700,000 Da. Sold under the name Betadine™, povidone–iodine is a very effective antimicrobial agent, more stable than tincture of iodine, and with the added advantages of lower irritancy and toxicity. Bacteria do not develop resistance to the preparation, it is active against *Chlamydia*, fungi, and viruses, including HIV and *Herpes*, and its sensitization rate of 0.7 % is low. Povidone–iodine is therefore widely used in medicine for skin cleansing pre- and postoperatively and for the prevention and treatment of infections of wounds, burns, ulcers, etc. The properties of PVP make it a useful agent for formulating many pharmaceutical and other products, and because statement of its inclusion in formulations is not always mandatory, its presence is frequently overlooked, a potential problem in some rare and hard to identify hypersensitivities.

Hypersensitivity responses to povidone–iodine include a few clear-cut cases of anaphylaxis, generalized urticaria–angioedema, and contact dermatitis, and, in almost all cases, PVP not iodine has been implicated as the offending component. Patch testing has been used to identify delayed reactions and for immediate responses skin prick testing is the diagnostic method of choice since it has been shown to perform well at concentrations of povidone–iodine 1 mg/ml (0.1 %) and PVP 35 mg/ml. Skin tests have generally been positive to povidone–iodine and PVP but not iodine, and in some other cases where PVP was present as an excipient with other drugs or agents, again PVP induced a positive response. It is therefore difficult to escape the conclusion that in the rare cases of hypersensitivity to preparations such as Betadine™, and perhaps in some other preparations where PVP has been included as a solubilizing, dispersing, suspending, or binding agent, as much attention should be directed at PVP as the preparation's active agent(s). There is a report of hypersensitivities to povidone–iodine where agents other than PVP seem to have been responsible. In a patch test study of suspected contact dermatitis induced by the antimicrobial agent, all ten patients reacted positively to povidone–iodine but no positive reactions were seen with PVP and one patient was positive to iodine. The authors did state, however, that it was difficult to distinguish between allergic responses and irritation.

Detection of IgE antibodies to PVP has been reported in a subject who experienced an immediate reaction that included urticaria and angioedema following topical application of povidone–iodine, but the weak reaction, interpreted as a positive result after simple adsorption of PVP to a microtiter plate, is not entirely convincing.

In summary, with povidone–iodine, the apparently inert substance PVP added to improve the formulation, rather than the active component, is likely to be the cause of cases of hypersensitivity occasionally seen with its use.

Summary

- Allergies to macrolide antibiotics are said to occur with an incidence of 0.4–3 %. Apart from the very occasional case report of anaphylaxis, the most commonly seen symptoms include urticaria, often generalized, angioedema, pruritus, asthma, tachycardia, and delayed cutaneous reactions presenting as maculopapular exanthema.
- Tetracyclines are viewed as being comparatively safe drugs. Minocycline, widely used for acne vulgaris, has been implicated in a serum sickness-like reaction, drug-induced lupus, cases of single organ dysfunction, and drug hypersensitivity syndrome reaction.
- Adverse reactions to rifamycins suggested to be immune mediated include a 'flu'-like syndrome, acute renal failure, hemolytic anemia, and thrombocytopenia. Other more typical manifestations of hypersensitivity include urticaria, contact dermatitis, erythema multiforme, and vasculitis.
- The most commonly occurring adverse effects caused by vancomycin are referred to collectively as red man syndrome. Reactions may range from mild pruritus, erythema, and flushing of the upper body to angioedema and rarely hypotension and cardiovascular collapse. Reactions may be prevented or their severity decreased by extending the infusion time and/or premedication with histamine H_1 and H_2 receptor antagonists.
- Despite its chemical similarity with vancomycin, teicoplanin does not appear to cause red man syndrome.
- Both rapid (carried out over several hours) and slow (over a period of days) desensitization protocols for vancomycin sensitivity have proved effective.
- Neomycin consistently ranks in the top 10 % of the most common causes of allergic contact dermatitis. Neomycin is also known to cause generalized reactions such as exfoliative dermatitis and erythroderma.
- Measured incidences of bacitracin sensitivity, as low as 0.3 % in 1973, increased to a lower range estimate of 1.5 % in the last 20 years but more recent estimates are consistently in the range 8–9.5 %.
- Some patients demonstrate contact allergy to both bacitracin and neomycin even though the two antibiotics have markedly different structures. Such co-sensitivities even extend to the cyclic peptide antibiotic polymyxin.
- Because of its greatly reduced usage, allergic contact dermatitis is currently not a problem with streptomycin. Reports on anaphylaxis to the antibacterial go back over 50 years with a relatively high incidence of reactions occurring in the 1960s.
- Delayed-type cutaneous reactions to clindamycin include pruritus, exanthematous rash, generalized maculopapular exanthema, erythroderma, generalized exanthematous pustulosis, and Stevens–Johnson syndrome.
- Many of the reactions to sulfonamides involve the skin and mucous membranes. The more severe reactions that occur include potentially lethal toxidermias and a delayed hypersensitivity-type syndrome characterized by fever, skin rash, and multi-organ toxicity.
- Immediate type I reactions are the most well-defined sulfonamide-induced hypersensitivity reactions with the best defined allergenic drug structures. Sulfonamides with one methyl substituent on a five- or six-membered aromatic heterocyclic ring on the carbon β to the sulfonamido substituent are the structures most complementary to anti-sulfamethoxazole IgE antibody combining sites.
- About 10 % of sulfamethoxazole is metabolized to a reactive hydroxylamine intermediate by CYP2C9 and myeloperoxidase. The unstable hydroxylamine intermediate auto-oxidizes to the highly reactive, toxic, immunogenic, and allergenic nitroso-sulfamethoxazole.
- Nitroso-sulfamethoxazole, but not the parent drug, can react covalently with cysteine residues of cellular surface proteins including skin keratinocytes, circulating peripheral blood mononuclear cells and splenocytes, and serum proteins, predominantly immunoglobulins and albumin, to form hapten–protein complexes. The hydroxylamine derivative may also be

involved in a number of adverse reactions including hepatitis, nephritis, thrombocytopenia, lupus erythematosis, and the sulfonamide hypersensitivity syndrome.

- Antigen-presenting cells alone may be sufficient to generate metabolites of sulfamethoxazole, produce the hapten–protein antigen complexes, and ultimately induce the T cell response to the drug.
- A serious drawback to the use of sulfamethoxazole–trimethoprim in HIV-infected patients is a high rate of adverse reactions that can range in frequency up to 50–60 % and require discontinuation of therapy.
- The presence of the sulfonamide group in a wide variety of frequently used non-antibacterial drugs raises the question of possible allergenic cross-reactivity between drugs with a common sulfonamide group. Based on the chemistry, metabolism, immune responses, and clinical findings, cross-reactions are thought not to occur. This conclusion also seems to apply to the sulfonylarylamine antiretrovirals amprenavir and fosamprenavir both of which contain a 4-aminobenzenesulfomamido group but nevertheless, clinicians should remain watchful.
- Almost all reports of trimethoprim-induced hypersensitivities are of the immediate kind. At least three different trimethoprim allergenic determinant structures complementary to the combining sites of trimethoprim-reactive IgE antibodies have been identified. One of the determinants, the 3,4-dimethoxybenzyl structure represents one-half of the trimethoprim molecule; the other two comprise structures that make up the entire, and almost the entire, molecule.
- Immediate hypersensitivity reactions that may involve skin rashes, pruritus, respiratory distress, and shock are the most frequently reported immune-mediated reactions to quinolones with a frequency of from 0.4 to 2 %. Skin testing to diagnose immediate allergies to quinolones is currently not widely and confidently accepted and practiced.
- IgE antibodies apparently specific for quinolones have been detected using a drug–Sepharose solid phase complex covalently linked with bis-oxirane. Quinolone-reactive IgE antibodies are found in some "normal" sera. This finding adds interest to the results showing positive skin tests in apparently normal, healthy controls.
- Although reactions with an immediate type I mechanism are the most important chlorhexidine-induced allergic reactions and receive most attention, delayed hypersensitivity reactions to the drug do occur and immediate and delayed reactions can occur in the same patient.
- A specific assay to detect IgE antibodies to the drug has been developed. The structure shown to be most complementary to the antibody combining sites was the entire chlorhexidine molecule.
- Hypersensitivity responses to povidone–iodine include a few clear-cut cases of anaphylaxis, generalized urticaria–angioedema, and contact dermatitis. Polyvinylpyrrolidone not iodine has been implicated as the offending component.

Further Reading

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Drugs and Other Agents Used in Anesthesia and Surgery

7

Abstract

Neuromuscular blocking drugs (NMBDs) are the most common cause of anaphylaxis during anesthesia representing ~60 % of reactions and an incidence of 1 in 1,000–20,000. Reactions are mediated by IgE antibodies with specificity for tertiary and quaternary ammonium ions, but adjoining structures may also be recognized. Recognition of the substituted ammonium groups accounts for the extensive cross-reactivity between the NMBDs. Diagnosis of reactions is effected by skin testing with free drugs, IgE antibody assays (especially using a morphine-solid phase), and the tryptase assay. Reversal of rocuronium-induced NM block with the cyclodextrin sugammadex has highlighted the question of changed allergenicity of such chemically sequestered drugs in host–guest complexes. Anaphylactic reactions to the hypnotics thiopentone and propofol are rare. Both are diagnosed by skin testing and the former also by a specific IgE test. True IgE-mediated reactions to local anesthetics are extremely rare; many reactions appear to be vasovagal responses, but delayed reactions are well known. Anaphylactic and other adverse reactions occasionally occur to colloids such as hydroxyethyl starch, gelatin, and dextrans, to the polypeptides protamine and aprotinin, and to heparins and patent blue V. Pre-injection of small MW dextran 1 reduces the incidence of dextran-induced anaphylaxis from 25 to 3 per 100,000.

7.1 Drug-Induced Reactions During Anesthesia

Although anesthesia today is safer than it has ever been, it still involves significant risk due to a number of factors including exposure of patients to a mixture of different drugs often given intravenously and over a short time period, systems failure, and the presence of abnormalities in some already sick patients. The administration of a variety of drugs that alter patients' normal physiological functions in a short time together with a range of symptoms, mild to severe, can make the task of pinpointing the cause of an adverse reaction a difficult one. Achieving this will depend on careful consideration of the drug(s) involved, the signs and symptoms of the patient's reaction, the temporal relationship of drug administration to

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_7, © Springer Science+Business Media, LLC 2013

symptoms, and the diagnostic tests employed. As well as application of appropriate investigative tests, the efficiency and effectiveness in classifying a reaction may depend on some factors that are not readily apparent. These include the education of clinicians in managing adverse reactions during and after the perioperative period, whether appropriate guidelines have been issued and followed and whether a suitable reporting process, spontaneous and/or statutory, is in place. Predictably, practices, guidelines, and regulations vary widely with some localities far in advance of others. In France for example, more attention is paid to the safety of anesthesia as a result of rare serious drug-induced adverse events and the reporting, registering, and subsequent investigation of such events is more organized than elsewhere. This well-organized strategy has enabled the regular and ongoing publication of data on substances responsible for anaphylaxis in France from 1984 to 2004 with more recent findings no doubt following soon. When information is needed or sought on epidemiological, clinical, and a number of associated features of reactions under anesthesia, the value of the French approach is apparent. However, whether the organization and arrangements are as developed and sophisticated as in France or carried out by only a few, or perhaps even a single individual, the interest and application of those in the profession of anesthesiology dedicated to keeping up with the ever-present problem of adverse reactions are essential.

Symptoms of a pseudoallergic reaction, for example, one resulting from direct histamine release, may give the same clinical picture as a true anaphylactic reaction, thus making it difficult to distinguish the two. The same clinical picture requires the same immediate management but, if for no other reason than patient safety during subsequent drug treatments and anesthesia, it is apparent that the underlying mechanism of any adverse reaction must be identified. This means establishing whether a drug-induced reaction during anesthesia is anaphylactic, that is, immune mediated, or anaphylactoid, where no immune basis for the reaction exists. This is a prime objective once successful treatment of the reaction has been effected. Data on the proportion of anaphylactic and anaphylactoid reactions from different surveys sometimes yield surprisingly different results, results that often originate in differences between a centralized recording and investigative methodology and a far less organized approach by a few individuals relying on spontaneous reporting of cases. In an example of the latter approach, the drugs responsible for adverse reactions in 350 consecutive patients during anesthesia were identified and the underlying mechanisms of the reactions were studied by intradermal testing, serum tryptase determinations, and drug-reactive serum IgE antibody tests. The reactions in 139 patients (39.7 %) were shown to be anaphylactic in nature, that is, evidence for an immunologic cause was found and the figures of 39.7 and 60.3 % for those experiencing a drug-induced anaphylactic and anaphylactoid reaction, respectively, are almost the exact reverse of the figures obtained in three French surveys where the corresponding results are 1999–2000 66 and 34 %, 2001–2002 69 and 31 %, and 1997–2004 72.2 and 27.8 %. This disparity may be a result of a reporting and investigative system that, in the French surveys, sorts and selects patients more likely to have experienced a true type I immediate reaction. While the French survey figures reflect organization and procedures that are preeminent for the study of anaphylaxis to drugs during anesthesia, they may not reveal the everyday situation of the wide range of adverse patient responses, many mild to moderately severe, to the variety of drugs administered. For drug reactions during anesthesia one of the first and most pertinent questions is what proportions of everyday adverse reactions are anaphylactic and non-anaphylactic (anaphylactoid)? Whether the proportion is 40:60 or 70:30 will depend on a number of factors, in particular, criteria and guidelines for reporting, selection, and testing and this should be remembered when considering drug allergy data collection and interpretation.

7.2 Incidences of Drug- and Other Agent-Induced Anaphylaxis During Anesthesia

With the above proviso in mind, the most comprehensive collection of data on drugs involved in immunoglobulin E-mediated reactions during anesthesia is contained in the ongoing French studies beginning with results of the 1984–1989 survey and in the long-standing Australian series gathered and maintained for over 30 years by Malcolm Fisher at the Royal North Shore Hospital of Sydney. In France, from 1984 until the most recently published survey that covers the years 1997–2004, 60–70 % of immediate hypersensitivity reactions that occur have been classified as true type I IgE antibody-mediated reactions. Mortality resulting from anaphylactic reactions during anesthesia is in the range of 3–9 % depending on the country. Neuromuscular blocking drugs (NMBDs) have consistently been the most common cause of anaphylaxis far exceeding the next most implicated agents latex, antibiotics, colloids, hypnotics, and opioids in that order (Table 7.1). Over the 20-year period of seven consecutive surveys the incidence of reactions to NMBDs has ranged from a high of 81 % in the 1984–1989 survey to a low of 58.2 % in the 1999–2000 survey, with the figure for 1997–2004 of 58.1 % being almost identical. IgE antibody-mediated reactions to latex (see Sect. 7.8.3) have shown upward movements since the first survey despite increasing awareness of the risk of latex sensitization in children with spina bifida and healthcare workers and the adoption of measures to minimize risk such as the upgraded requirements for surgical gloves and making surgery a latex-safe environment. The incidence of type I reactions to antibiotics also markedly increased from only 2 % of the total in 1984–1989 to 15.1 % in the 1999–2000 survey. Allergic reactions to antibiotics appear to be increasing with time with penicillins and cephalosporins (see Chap. 5) accounting for most of the increase. Comparing the Australian survey involving 606 patients with

Table 7.1 Agents responsible for type I immediate allergic reactions during anesthesia

Agent	Reaction (%) France[a]	Reaction (%) Australia[b]
Neuromuscular blocking drugs	**58.1**	**61.9**
Succinylcholine	33.4	32.8
Rocuronium	29.3	16.8
Atracurium	19.3	9.1
Vecuronium	10.2	5.6
Pancuronium	3.6	1.9
Mivacurium	2.5	0.5
Cisatracurium	1.7	0.5
Alcuronium[c]		24.8
d-Tubocurarine		2.9
Gallamine		2.1
More than one drug[d]		2.1
Hypnotics/Induction agents	**2.3**	**10.4**
Propofol	55.8	6.3
Midazolam	32.6	52.4
Thiopentone	9.3	30.2
Ketamine	2.3	9.5
Alfathesin		1.6
Propanidid		
Methohexitone		
Latex	**19.7**	**0.8**
Antibiotics	**12.9**	**8.6**
Penicillins	49.0	15.4
Cephalosporins	37.0	73.1
Vancomycin		5.8
Others	14.0	5.8
Colloids	**3.4**	**4.6**
Gelatin	89.9	85.7
Hetastarch	9.5	
Albumin	1.6	
Dextran 70		14.3
Opioids	**1.7**	**2.6**
Morphine	35.5	50.0
Fentanyl	22.6	25.0
Sufentanil	22.6	
Nalbuphine	12.9	
Remifentanil	6.5	
Meperidine (Pethidine)		18.7
Omnopon		6.3
Other agents[e,f]	**2.7**[e]	**3.8**[f]
No causal drug detected		7.4

[a]Survey in France 1997–2004; 1,816 patients. Data from Mertes PM et al. J Allergy Clin Immunol. 2011;128:366

[b]Ongoing Australian survey; 606 patients. Data from Fisher MM et al. Acta Anaesthesiol Scand. 2011;55:99

[c]Discontinued

[d]Eight reactions with two different neuromuscular blocking drugs administered

[e]Made up largely of patent blue, propacetamol, local anesthetics, aprotinin, and protamine

[f]Made up largely of induction agent plus neuromuscular blocker (four patients), protamine, local anesthetics, patent blue, chlorhexidine, contrast media, and ondansetron

the French series, three features in particular are noteworthy. NMBDs again predominate with an incidence close to the French figure, reactions to hypnotics, principally due to the induction agents thiopentone and alfathesin, are markedly higher in the Australian figures and the difference in the incidence of latex anaphylaxis is remarkable (Table 7.1). Within the NMBDs, the surveys agree that succinylcholine accounts for a third of reactions, but otherwise there are some striking differences. Rocuronium, considered by some in Europe to be a risk for anaphylaxis (see Sect. 7.4.4.3) and introduced in 1994, in other words, many years after succinylcholine, still accounted for nearly 30 % of reactions in France. The Australian incidence is almost half that, but alcuronium (see Sect. 7.4.4.3), now off the market for nearly two decades, represents almost one-quarter of the 375 Australian reactions to NMBDs.

Both surveys for reactions to antibiotics reveal the β-lactam antibiotics to be the dominant culprit drugs with incidences of 86 and 88.5 % in the French and Australian figures, respectively. However, while penicillins were implicated a little more often than cephalosporins in the French experience (49–37 % of reactions to antibiotics), the number of reactions to cephalosporins in Australian patients was nearly five times as great as the penicillins figure (73.1–15.4 %).

Adverse reactions to the penicillin and cephalosporin antibiotics, two of the clinically most important families of antimicrobial drugs but also two of the most allergenic, are examined in detail in Chap. 5 and will not be discussed further here. The opioid group of drugs are responsible for around 2 % of reactions during the perioperative period. These histamine-releasing, clinically important analgesics are dealt with separately in Chap. 8.

7.3 Clinical Features of Anaphylactic and Anaphylactoid Reactions During Anesthesia

Symptoms of anaphylaxis and an anaphylactoid response are often sufficiently similar to make distinguishing them difficult and symptoms alone should not be relied upon in making a diagnosis. However, symptoms in anaphylactic patients do seem to be more severe—on a graded scale of clinical manifestations most anaphylactic reactions during anesthesia fall into grades 2 and 3 (see Sect. 2.2.1.1.4) while anaphylactoid reactions are mainly grade 1. Most anaphylactic reactions during anesthesia occur within minutes of induction and, mainly, occur following intravenous administration of the drug or agent. For both anaphylactic and anaphylactoid episodes, adverse cardiovascular reactions are a common and serious symptom which may progress to cardiovascular collapse if not treated. Multi-organ involvement is usually the case, but for anaphylactic patients in particular, cardiovascular collapse may be the only symptom with frequencies of occurrence of up to more than 80 % and the sole feature in up to 60 % of cases. As with cardiovascular symptoms, bronchospasm is more often seen in anaphylaxis, but the incidence of cutaneous symptoms is far greater in anaphylactoid reactions (Table 7.2). In one survey, bronchospasm was seen in 19 % of patients who experienced anaphylaxis during anesthesia and it was the sole clinical feature in 4.5 % of patients. Findings from a multivariant analysis placed in a clinical context suggest that a patient with bronchospasm and hypotension in the perioperative situation is 27 times more likely to prove skin

Table 7.2 Clinical features of anaphylactic and anaphylactoid reactions during anesthesia

Symptoms	Anaphylactic reactions (%)[a]	Anaphylactoid reactions (%)[b]
Cardiovascular	74.7	33.9
Arterial hypotension	17.3	18.4
Cardiovascular collapse	50.8[c]	11.1
Bradycardia	1.3	0.7
Cardiac arrest	5.9	0
Bronchospasm	39.8	19.2
Cutaneous	71.9[d]	93.7[e]
Angioedema	12.3	7.7

Reactions in France 1999–2000
Data from Mertes PM et al. Anesthesiology 2003; 99: 536
[a]518 anaphylactic patients
[b]271 anaphylactoid patients
[c]Sole feature in 6.2 % of cases
[d]Sole feature in 9.7 % of cases
[e]Sole feature in 50.2 % of cases

test positive compared to those who do not develop hypotension, and bronchospasm in anaphylactic patients is more likely to be severe than in those patients where the bronchospasm was provoked by a nonimmune mechanism. The role of direct histamine release in the etiology of bronchospasm during anesthesia is unclear since although the autacoid exerts a powerful effect on the bronchi when inhaled in nebulized form, its effects are far less obvious when it enters the general circulation. It therefore seems likely that the histamine-releasing capacities of anesthetic drugs as a factor in the induction of bronchospasm are not as important as sometimes assumed and expressed. For an expanded discussion of direct histamine release by drugs, see Chap. 8.

7.4 Anaphylaxis to Neuromuscular Blocking Drugs

In 1942 Griffith and Johnson in Montreal introduced their landmark paper (Anesthesiology 1942; 3: 418) on the first use of drug-induced muscle relaxation in anesthesia with the statement—"Every anesthetist has wished at times that he might be able to produce rapid and complete muscular relaxation in resistant patients under general anesthesia." Using what was described as a "purified curare" preparation or "Intocostrin" (Extract of Unauthenticated Curare, Squibb), Griffith and Johnson administered the preparation intravenously at a dose of 10–20 mg per 20 lbs body weight to 25 patients and obtained temporary but complete muscular relaxation with no apparent harmful effects. They concluded: "…curare may prove to be a drug which will occasionally be of great value, and will give us a means of providing the surgeon rapidly with excellent muscular relaxation at critical times during certain operations." Within a year of the initial report, the "curare" preparation, essentially an early purification of *d*-tubocurarine, had been used in 131 patients during general anesthesia and in 1952 Foldes et al. (N Engl J Med 1952; 247: 596) summed up a decade's use of muscle relaxants in anesthesia in the following words—"…[the] first use of muscle relaxants in anesthesiology by Griffith and Johnson in 1942 not only revolutionized the practice of anesthesia but also started the modern era of surgery and made possible the explosive development of cardiothoracic, neurological and organ transplant surgery."

7.4.1 Some Epidemiological Background

Most case series, surveys, and diagnostic and mechanistic studies on NMBDs, the most frequent instigators of drug-induced anaphylaxis during anesthesia, have been undertaken in Australia, France, New Zealand, the United Kingdom, and, more recently, Scandinavia and Spain. Few investigations have been pursued in the USA where reactions are presumably rare or go unrecognized or unreported. Incidences of reactions vary between countries and estimates have ranged from 1 in 1,000–2,000 to about 1 in 20,000. In the United Kingdom about 500 reactions a year are thought to occur while the incidences in France, Norway, and Australia are said to be ~1 in 5,500, 1 in 5,200, and 1 in 10,000, respectively. With regard to prior sensitization of reactors, estimates of previous exposure to an NMBD range from about 15 to 50 % and atopy does not seem to be a significant risk factor. A history of previous anesthesia is also not a risk factor, but a history of an adverse reaction during a previous anesthesia is. Many studies over the last 30 years have concluded that female reactors predominate with ratios compared to males of up to four to one. The median annual incidences of allergic reactions to NMBDs estimated in a 1997–2004 French survey were 105.5 and 250.9 per million procedures for men and women, respectively. The overall annual incidence per million for both sexes was 184 and for children, males and females showed an equal incidence of 61 cases. The number of children sensitized to NMBDs was found to increase with adolescence. The distribution of anaphylactic reactions according to age for adults showed peaks for males in the 10–20 and 40–50 years age ranges, and for females, the highest incidences occurred between 30 and 60 years with the peak in the 40–50 year range.

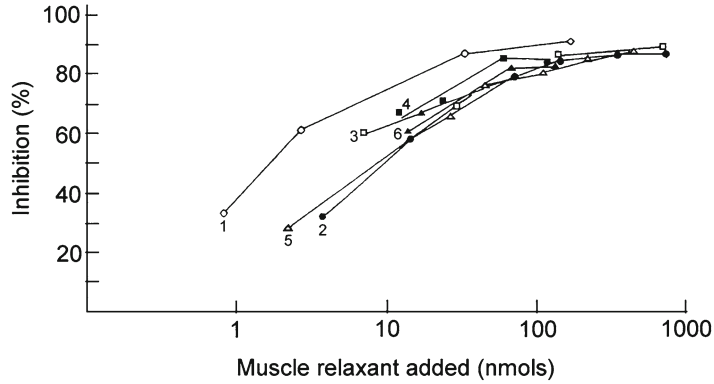


Fig. 7.1 Demonstration of allergenic cross-reactivity between NMBD. Inhibition by six different NMBDs of the binding of IgE antibodies in the serum from an alcuronium allergic patient to an alcuronium-solid phase conjugate. Key: (1) alcuronium (*open circle*); (2) *d*-tubocurarine (*filled circle*); (3) succinylcholine (*open square*); (4) decamethonium (*filled square*); (5) gallamine (*open triangle*); (6) pancuronium (*filled triangle*). From Baldo BA, Fisher MM. Anaphylaxis to muscle relaxant drugs: Cross-reactivity and molecular basis of binding of IgE antibodies detected by radioimmunoassay. Mol Immunol.1983;20:1393. Reprinted with kind permission from Elsevier Limited

7.4.2 Mechanisms Underlying Anaphylaxis to Neuromuscular Blocking Drugs

For information on histamine and its metabolism, see Sect. 3.2.5.1.

7.4.2.1 Immunoglobulin E Recognition of Allergenic Determinants

The first reports of anaphylactic-like reactions to NMBDs appear to have been in the late 1960s, and by the early 1980s skin testing with the free drugs was being routinely used to diagnose reactions, identify sensitivity to the drugs, and look for cross-reactivity between the different NMBDs. Utilizing sera taken from patients who experienced an anaphylactic reaction to an NMBD, Baldo and Fisher in 1983 developed a radioimmunoassay to demonstrate the presence of serum IgE antibodies to a solid phase covalent complex of alcuronium. Quantitative binding and hapten inhibition studies demonstrated that antibodies to one NMBD generally also recognized and reacted with other NMBDs (Fig. 7.1, Table 7.3). Also, results with panels of carefully selected drugs and chemicals with no muscle relaxant properties examined in inhibition experiments showed that the cross-reactive antibody-binding structures on the NMBDs were quaternary and tertiary ammonium ions, the former groups being the structure involved in conferring neuromuscular blocking activity on the muscle relaxant drugs. Apart from the presence of ammonium ions, some of the cross-reacting drugs showed little or no structural similarity. Drugs with diverse pharmacological activities were inhibitory including an antihistamine, a neuroleptic, a ganglionic blocking agent, an opioid analgesic, an acetylcholine

Table 7.3 Serological cross-reactivity between neuromuscular blocking drugs

Neuromuscular blocking drug	Amount (nmol/tube) for 60 % inhibition of binding[a]
Alcuronium	1.5
Succinylcholine	7.0
Decamethonium	9.0
Gallamine	16.5
d-Tubocurarine	17.0
Pancuronium	14.0

Serum from a patient who experienced anaphylaxis to alcuronium
Data from Baldo BA, Fisher MM. Mol Immunol 1983;20:1393
[a]Inhibition of binding to an alcuronium-solid phase

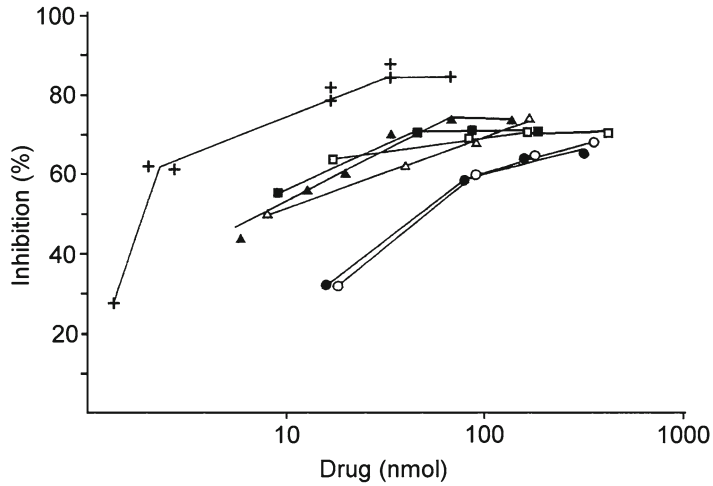


Fig. 7.2 Cross-reactivity of NMBD-reactive IgE antibodies with some widely used drugs of different pharmacological action but which all contain quaternary and/or tertiary ammonium groups. Inhibition of alcuronium-reactive IgE antibody binding to an alcuronium-solid phase by alcuronium (*plus sign*); promethazine HCl (*open circle*); chlorpromazine HCl (*filled circle*); neostigmine bromide (*open square*); pentolineum tartrate (*filled square*); trimethaphan camphorsulfonate (*open triangle*); morphine HCl (*filled triangle*). From Baldo BA, Fisher MM. Anaphylaxis to muscle relaxant drugs: Cross-reactivity and molecular basis of binding of IgE antibodies detected by radioimmunoassay. Mol Immunol.1983;20:1393. Reprinted with kind permission from Elsevier Limited

Table 7.4 Recognition of some frequently used drugs of diverse pharmacological activities by neuromuscular blocking drugs-reactive IgE antibodies

Drug	Amount (nmol/tube) of drug needed for 60 % inhibition of binding[a]
Alcuronium	2.0
Promethazine HCl	90
Chlorpromazine HCl	90
Neostigmine bromide	<17
Pentolineum tartrate	15
Trimethaphan camphorsulphonate	30
Morphine HCl	19

IgE antibodies in serum from a patient who experienced anaphylaxis to alcuronium. Serum is the same as used in inhibitions with neuromuscular blocking drugs—see results Table 7.3

Data from Baldo BA, Fisher MM. Mol Immunol 1983;20:1393

[a]Inhibition of binding to an alcuronium-solid phase

receptor antagonist, and an acetylcholinesterase inhibitor (Fig. 7.2, Table 7.4). Quaternary ammonium compounds including some tetraalkylammonium bromides and a series of alkyltrimethylammonium salts in inhibition studies were generally clearly recognized by the NMBD-reactive IgE antibodies showing different inhibitory potencies with different sera. For example, in the results shown (Fig. 7.3, Table 7.5), the tetraalkylammonium salt octyltrimethylammonium bromide was almost equally potent in inhibiting the IgE in the patient's serum as the NMBD implicated in the patient's anaphylactic reaction and, on a molar basis, was over three times as potent as the ethyl and dodecyl derivatives and nearly five times as potent as the hexadecyl derivative. These results suggested that as the alkyl chain increased in length, the optimum "fit" for the complementary antibody combining sites was reached with a chain length of around eight carbons (bearing in mind that chain lengths of 3–7 and 11 carbons were not tested), and with the addition of more carbons, inhibitory activity declined. Also, as one might expect, the nature of the alkyl group within the ammonium ion is important for antibody recognition. Of the three tetraalkylammonium salts tested, the tetramethylammonium

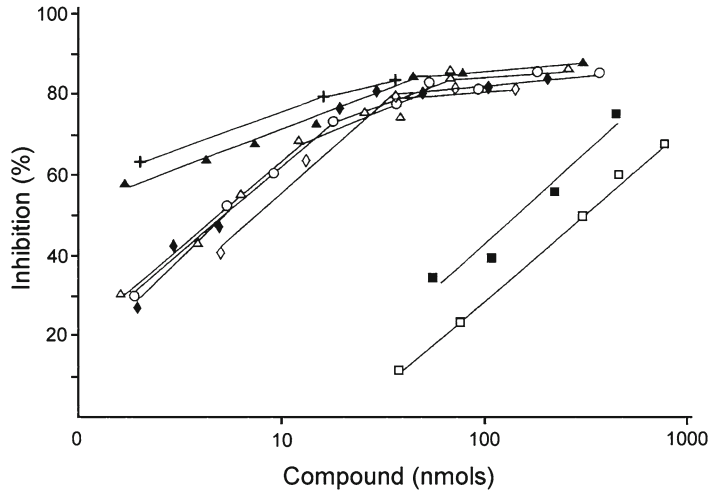


Fig. 7.3 Cross-reactivity of NMBD-reactive IgE antibodies with quaternary ammonium compounds. Inhibition by tetraalkylammonium and alkyltrimethylammonium salts of the binding of alcuronium-reactive IgE antibodies to an alcuronium-solid phase conjugate. The serum was from a patient who experienced anaphylaxis to alcuronium and is the same serum used in experiments summarized in Figs 7.1 and 7.2. Key: alcuronium (*plus sign*); tetramethylammonium bromide (*open circle*); tetrapropylammonium bromide (*filled square*); tetrapentylammonium bromide (*open square*); ethyltrimethylammonium bromide (*open triangle*); octyltrimethylammonium bromide (*filled triangle*); dodecyltrimethylammonium bromide (*filled diamond*); hexadecyltrimethylammonium bromide (*open diamond*). From Baldo BA, Fisher MM. Anaphylaxis to muscle relaxant drugs: Cross-reactivity and molecular basis of binding of IgE antibodies detected by radioimmunoassay. Mol Immunol.1983; 20:1393. Reprinted with kind permission from Elsevier Limited

Table 7.5 Recognition of quaternary alkyl ammonium salts by neuromuscular blocking drug-reactive IgE antibodies

Quaternary ammonium compound	Amount (nmol/tube) needed for 60 % inhibition of binding[a]
Alcuronium (neuromuscular blocking drug)	<2.0
Ethyltrimethylammonium bromide	8.1
Octyltrimethylammonium bromide	2.5
Dodecyltrimethylammonium bromide	8.6
Hexadecyltrimethylammonium bromide	12.2
Tetramethylammonium bromide	8.6
Tetrapropylammonium bromide	230
Tetrapentylammonium bromide	500

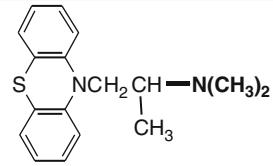
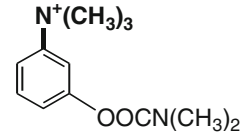
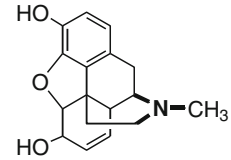
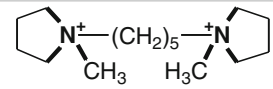
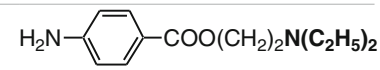
IgE antibodies in serum from a patient who experienced anaphylaxis to alcuronium. Serum is the same as used in inhibitions with neuromuscular blocking drugs—see results Table 7.3
Data from Baldo BA, Fisher MM. Mol Immunol 1983;20:1393
[a]Inhibition of binding to an alcuronium-solid phase. Results for alcuronium shown in Tables 7.3, 7.4 and 7.5 were obtained in three separate studies

salt was ~27 and 58 times as active as the tetrapropyl and tetrapentyl derivatives, respectively (Fig. 7.3, Table 7.5). Table 7.6 summarizes examples of compounds without neuromuscular blocking properties that are recognized and interact with NMBD-reactive IgE antibodies in sera from NMBD-allergic patients.

7.4.2.2 Fine Structural Specificities of IgE Antibodies that React with Neuromuscular Blocking Drugs

Extensive IgE antibody combining site specificity studies employing sera from patients with NMBD-induced anaphylaxis together with all

Table 7.6 Examples of some drugs and chemicals recognized by IgE antibodies that react with neuromuscular blocking drugs

Compound	Structure of compound or cation group with ammonium group highlighted[a]
Trialkylamines	**NR_3**[b]
Tetraalkylammonium salts	**N^+R_4**[c], [d]**$RN^+(R')_3$**[e]
Choline	**$(CH_3)_3N^+$**CH_2CH_2OH
Acetylcholine	**$(CH_3)_3N^+$**$CH_2CH_2OCOCH_3$
Promethazine	
Neostigmine	
Morphine	
Pentolineum	
Procaine	

From Baldo BA, Pham NH. Structure–activity studies on drug-induced anaphylactic reactions. Chem Res Toxicol 1994; 7: 703. Reproduced with permission from American Chemical Society

[a]Determinants show heterogeneity. They may be solely the ammonium group or extend to attached or nearby atoms or groupings. The exact confines of IgE-binding determinants are not always clear and depend on the particular serum (IgE) studied

[b]R = methyl or ethyl

[c]R = methyl, ethyl, propyl, etc.

[d]R = methyl, ethyl, …, hexadecyl, etc.

[e]R′ = methyl, ethyl, propyl, etc.

available NMBDs and selected analogs led to the conclusion that the combining site specificities of NMBD-reactive IgE antibodies fall into five main groups (Fig. 7.4):

Category 1. Antibodies that react with the ammonium group(s) of only one NMBD and are inhibited by that NMBD only. It is assumed that the specificity of such antibodies is precisely complementary to some fine structural detail(s) present uniquely on the group(s) of the NMBD (see for example discussion on alcuronium and rocuronium, Sect. 7.4.4.3).

Category 2. Antibodies with recognition profiles confined to the ammonium groups but which cross-react with, and are inhibited almost equally well by, each of the NMBDs with the same or similar groups linked to the nitrogens. Examples include succinylcholine and decamethonium; *d*-tubocurarine and atracurium; and pancuronium and vecuronium. However, since differences occur in the structures attached to the nitrogens in some NMBDs, antibodies to one NMBD, for example, succinylcholine, may not readily cross-react with, and be inhibited by, some other NMBDs, for example, rocuronium.

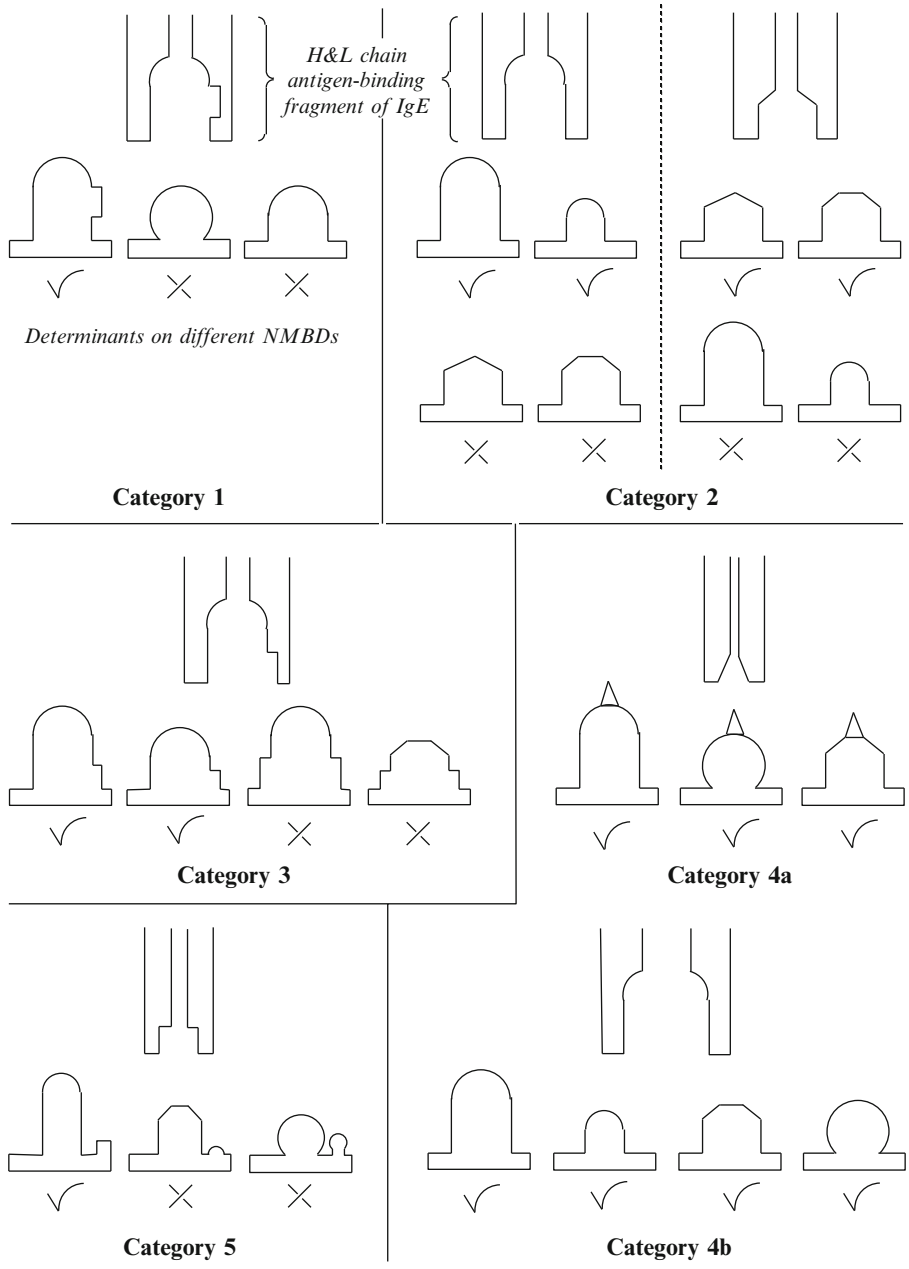


Fig. 7.4 Diagrammatic representation of different possible fine structural recognition patterns of IgE antibodies that react with neuromuscular blocking drugs. Category 1. Antibodies that react with the ammonium group(s) of only one NMBD. Category 2. Antibodies with recognition profiles confined to the ammonium groups but which cross-react with each of the NMBDs with the same or similar groups linked to the nitrogens. Category 3. Antibodies that recognize ammonium groups together with adjoining and/or adjacent structures. Category 4. Highly cross-reactive antibodies. Some antibodies may be promiscuous in their recognition profile, reacting with all or most NMBDs via a small combining site complementary to a small determinant structure present on a number of NMBDs (4a) or via a large combining site able to accomodate similar (but not necessarily identical) structures on different NMBDs (4b). Category 5. Antibodies that recognize a structure on an NMBD other than the ammonium ions that is not found on other NMBDs

Category 3. Antibodies that recognize ammonium groups together with adjoining and/or adjacent structures. Here inhibitory activities of different NMBDs may vary widely depending on the ammonium group and the uniqueness or otherwise of the neighboring structure(s) recognized.

Category 4. Highly cross-reactive antibodies. Some antibodies may be promiscuous in their recognition profile, reacting with all or most NMBDs. This may occur in two ways: (a) via recognition of a small fine structural feature common to all NMBDs or (b) via antibodies with larger combining sites lacking recognition of common fine structural detail but showing broad, less precise recognition of larger similar general structural features found an all or most NMBDs.

Category 5. Antibodies that recognize a structure on an NMBD other than the ammonium ions that is not found on other NMBDs.

So far, we have found only one serum containing IgE antibodies that reacted with, and were inhibited by, only one NMBD, but because the precise structure recognized remained unidentified, it was not possible to say whether the antibodies belonged to category 1 or category 5. Figure 7.4 sets out simplified diagrammatic representations of these five different antibody recognition profiles. It does not attempt to depict finer structural differences referred to. Given the known heterogeneity of antibody combining sites, some sera may contain mixtures of specificities from one or more of the five different categories.

7.4.2.3 Neuromuscular Blocking Drug-Induced Release of Mediators of Anaphylaxis

Simply binding specifically to IgE antibody combining sites is not enough by itself to describe a molecule as an "allergen." For an immediate, type I reaction, 'allergenic activity' is the property of specifically provoking an allergic response via the release of the biologically active molecules that cause the signs and symptoms of a hypersensitivity reaction and a true allergen has this property as well as IgE antibody-binding capacity. In anaphylactic responses such as those induced by NMBDs, mediators of the allergic reaction are released by the cross-linking of FcεRI-bound IgE molecules on mast cells and basophils. This occurs by allergen molecules reacting with the combining sites of the divalent antibody molecules, but to do this an allergenic molecule must be at least divalent. Even without being protein bound, allergenic divalency (or greater) is a requirement that all NMBDs fulfill by virtue of the substituted ammonium ions present in the molecules. The optimum molecular length of NMBDs, depolarizing or not, is 2.0–2.1 nm with the ten-carbon 2.0 nm length of decamethonium showing potent activity. The ammonium ions in NMBDs are, of course, responsible for the neuromuscular blocking property of the drugs, and at a distance apart of about 1–1.6 nm, bridging of adjacent antibody molecules can be effected. Experimental verification of this predicted free drug-induced release from human leukocytes has been provided. In in vitro histamine release studies comparing the NMBDs succinylcholine and pancuronium with a series of diammonium salts of increasing chain length, lengths greater than 4 Å (0.4 nm) were required for release to occur and a length of 0.6 nm produced optimum release. In addition, and as might be expected, NMBDs with rigid structures such as pancuronium were less active in promoting the release of histamine than flexible straight chain molecules like succinylcholine with its widely spaced terminal determinant groups. For a further discussion of allergen bridging of cell-bound IgE molecules for mediator release, see Sect. 3.1.2.

7.4.2.4 Inhibition of Histamine-N-Methyltransferase by NMBDs

All NMBDs inhibit histamine-*N*-methyltransferase (HMT), the primary catabolic enzyme for histamine in humans (see Fig. 3.6). Inhibition is competitive with respect to the methyl donor and noncompetitive with respect to histamine. Six different NMBDs, alcuronium, pancuronium, *d*-tubocurarine, gallamine, succinylcholine, and decamethonium, inhibited enzyme activity in the concentration range 10^{-7}–10^{-3} M with alcuronium being the most potent inhibitor ($ID_{50} = 2 \times 10^{-6}$ M)

and with the activity of the other NMBDs following in the above-listed order. Alcuronium proved to be of similar potency to the dimaprit analog SKF91488, one of the most potent HMT inhibitors known. In an investigation of structure–activity relationships of potent inhibitors of HMT, alcuronium was compared to the antimalarials amodiaquine, quinacrine, and chloroquine and to the antiseptic agent chlorhexidine. Each of the compounds showed marked similarities to the conformations of the arrangement of the three nitrogen atoms of histamine. Following the release of vecuronium, it was shown to be a better inhibitor ($K_i = 1$) than pancuronium ($K_i = 2$). Calculations showed that administration of vecuronium in doses of 0.1–0.15 mg/kg could inhibit HMT for up to 14 min by 25–50 %. This may explain the occasional severe bronchospasm seen in some patients given vecuronium.

7.4.3 Diagnosis of Anaphylaxis to Neuromuscular Blocking Drugs

A challenge (provocation) test with an NMBD for the diagnosis of allergic sensitization to NMBDs is of course not acceptable, so this means that other diagnostic methods must be relied upon and, for preference, the employment of more than one method is desirable. Because most, if not all, patients who experience an anaphylactic reaction to an NMBD are not allergically sensitized by prior exposure to one of these drugs, one can assume that the NMBD-reactive IgE antibodies were already present in the patients' sera prior to their anesthesia. This means that sensitization probably occurs through exposure to a stimulating allergen source or perhaps via a "natural" antibody (see below, Sect. 7.4.5.3) and one might therefore predict that the presence of such antibodies in the general population might not necessarily be rare. In fact, this appears to be the case. A study to determine the prevalence of NMBD reactivity in a sample of the general population in France showed that 9.3 % of 258 subjects had either a positive skin test to one or more NMBDs or the presence of quaternary ammonium ion-reactive serum IgE. Another relevant study in Scandinavia revealed that ~5 % of blood donors and 10 % of allergic subjects had serum IgE antibodies that reacted with a morphine solid phase (for the significance of this, see Sects. 7.4.3.4.2 and 7.4.5.2). These findings need to be kept in mind when skin tests and serum IgE antibody tests are being used to investigate suspected NMBD hypersensitivities.

7.4.3.1 Persistence of IgE Antibodies to Neuromuscular Blocking Drugs

In six patients investigated by skin testing and detection of serum IgE antibodies from 4 to nearly 30 years after NMBD-induced anaphylaxis during anesthesia, the positive skin test to an NMBD(s) obtained soon after the reaction was found to persist while NMBD-reactive antibodies were detected in the five patients tested. In a study of changes over a 4–13 year period of intradermal test results, the positive tests for an NMBD(s) incriminated at the time of the reaction remained positive in 15 of 18 patients. An investigation of changes in NMBD-reactive IgE antibodies in seven NMBD-allergic patients showed six had persisting positive tests with the antibody levels increasing in one patient and decreasing in five. Results with one patient were interesting and somewhat unexpected. After initially reacting to decamethonium and testing skin test positive to this drug, negative to succinylcholine, and positive to succinylcholine in the IgE immunoassay, all of the tests proved positive 5 years later, but after a further 2 years the intradermal test for succinylcholine was negative while the serum IgE antibody test for the drug remained positive. In many direct binding IgE antibody and inhibition studies employing the full range of available NMBDs, reactivities of decamethonium and succinylcholine invariably seemed to go together. This suggested that the patient's sensitivity to decamethonium/succinylcholine detected by the antibody immunoassay was persisting, and although skin testing did not pick it up, the wisest advice was to avoid the responsible drugs for the rest of the patient's life. Skin tests, leukocyte histamine release experiments, and IgE antibody immunoassays were utilized to investigate persistence of allergy to NMBDs in 21 patients who reacted to succinylcholine. Skin tests showed sensitization to the drug persisted in 18 patients 1–4 years later,

and histamine release and IgE determinations also remained positive in most of the patients. Seasonal variations to allergens such as pollens and other investigations involving a variety of different allergens have shown that IgE antibody production increases with antigen exposure, but some observations such as the transfer of allergy in humans by bone marrow transplants show that cells retain the capacity to secrete specific IgE antibodies in the absence of continuing exposure. This is supported by the demonstration of immunoglobulin E secreting cells in both the marrow and lymph nodes draining the site of antigenic challenge in laboratory animals.

7.4.3.2 Serum Tryptase Determination

The tryptase test is discussed in detail in Sect. 4.5.1 where details of the assays for total and mature tryptase are given.

Upon the induction of what appears to be an anaphylactic-like response during anesthesia and once the reaction has been brought under control and the patient stabilized, the primary question must be was the reaction immune mediated, that is, anaphylactic, or nonimmune, that is, anaphylactoid in nature? Although elevation of the concentration of mature and total (>11.4 μg/l) tryptase in serum is not always a certain indication of a true anaphylactic reaction since its presence, like histamine, can sometimes be released from mast cells via nonimmunologic mechanisms, an increased tryptase level suggests that the reaction is more likely to be IgE antibody mediated and the greater the increase the higher the probability that the reaction is anaphylactic rather than anaphylactoid. It should also be pointed out that the release of tryptase by nonimmune mechanisms is not unequivocally established since serum tryptase levels are not, for example, increased during vancomycin-induced anaphylactoid reactions even though the drug induces tryptase release from mast cells in vitro (see also, Sect. 6.1.4.2). In summary, the in vivo serum tryptase test result, whether it is positive or negative, provides invaluable information to be considered along with other important test results, in particular skin test and drug-specific IgE antibody determinations, in efforts to establish a diagnosis and identify the underlying mechanism of a suspected drug-induced reaction during anesthesia.

7.4.3.3 Skin Tests

For a detailed discussion of the employment of skin testing in the diagnosis of drug allergy, the reader is referred to Sect. 4.2.

There is general agreement on the value of skin testing with NMBDs for the diagnosis of allergic sensitivity to the drugs but, as mentioned, the so-called gold standard of challenge with the drug delivered in incremental doses cannot be done so it is not possible to validate the test. Despite this, the positive and negative predictive value of the skin test appears to be good even without validation. In one recent diagnostic investigation of rocuronium allergy the authors considered diagnosis was established by a positive skin test for the drug without further tests. Skin testing then is the preeminent diagnostic test for NMBD-allergic sensitivity, but therein lays the nagging doubt that some critics have for the test. False-positive results with prick tests, and more likely with intradermal tests, occur and some believe that in the diagnosis of anaphylaxis to NMBDs false positives occur more often than they should and sometimes this is not recognized. In particular, doubts have been expressed about the concentrations of rocuronium and vecuronium recommended for prick testing and intradermal testing. As well as false positives, negative skin tests have occasionally led to an anaphylactic reaction to a second NMBD but the end result is that whenever a false positive skin test or a failure to detect an allergic sensitivity occurs, the culprit drug and the underlying mechanism remain unidentified.

7.4.3.3.1 Methodology

Skin testing of patients who experienced a suspected immediate allergic reaction to an NMBD should be undertaken no earlier than 4–6 weeks after the reaction. Since most of the IgE antibodies may have been consumed during the allergic event, testing within the 4–6 week period increases the risk of a false-positive finding, so only positive results should be taken notice of and a negative test should be repeated after the

recommended delay. For prick testing, the commercially available preparations of the NMBDs are used diluted if necessary with sterile physiological saline. If a bacteriostatic is required phenol 0.5 % w/v may be added. A positive histamine control (10 mg/ml in saline) and a negative (vehicle) control are always included. Information on the shelf life of diluted NMBD preparations is lacking, an indication that solutions for skin testing should be freshly prepared at the time of testing and rejected upon completion but, except for atracurium, cisatracurium, mivacurium and rocuronium, each of which should be freshly diluted, storage of test solutions for up to 3 months at 4 °C has been suggested by some. The NMBD solutions are used diagnostically on the forearm or back in skin prick and intradermal testing; the former is a little more specific but less sensitive, that is, it has a small tendency to give false negative results while the latter test is more sensitive but less specific, that is, it produces more false positives. Up to 97 % concordance and a similar diagnostic value have been found in studies comparing the two methods. A commonly used prick test procedure employs side by side the commercial NMBD injectable solutions neat and diluted one in ten, the latter to reduce false-positive reactions. Atracurium, mivacurium, and rocuronium are more likely to provoke histamine-induced nonspecific wheals leading some to recommend starting their testing at a one in ten dilution. A wheal diameter of at least 2 mm is generally taken as a positive test, but because NMBDs often produce smaller wheals than common inhalant and other allergens, a positive wheal to neat test solution but a negative to the one in ten dilution may be considered diagnostic. In a slightly different approach, the skin prick test is used to guide the choice of the first concentration selected for intradermal injection. In this protocol issued by the French Society for Anaesthesia and Intensive Care (SFAR) and French Society of Allergology (SFA), a positive prick test is defined as a wheal with a diameter 3 mm greater than the negative control or a diameter at least half that of the positive control 20 min after completion of the test. When the prick test is negative, a 1 in 1,000 dilution of the commercial NMBD solution is used to initiate intradermal testing and thereafter ten times stronger concentrations (up to the maximum concentration) of solution are used at 20 min intervals until a positive reaction is seen. The maximum concentration should not be exceeded. Some other practitioners carry out intradermal testing at the highest concentration of NMBD that does not cause a reaction in normal subjects. For intradermal testing 0.02–0.05 ml of solution is injected into the dermis to give a bleb of ~4 mm diameter. The SFAR-SFA criterion for a positive reaction is the appearance after 20 min of an erythematous wheal with a diameter at least twice that of the original bleb. Table 7.7 lists the concentrations of NMBDs for skin prick and intradermal testing together with criteria for a positive test in guidelines issued by the SFAR and SFA. The reliability of prick testing using the recommended concentrations for rocuronium (10 mg/ml undiluted) and vecuronium (4 mg/ml undiluted) has been questioned following the

Table 7.7 Concentrations[a] of neuromuscular blocking drugs used for skin testing

Drug	Skin prick test[b] concentration (mg/ml)	Intradermal test[c] concentration (μg/ml)
Succinylcholine	10	100
Rocuronium[d]	10	50
Vecuronium[d]	4	400
Pancuronium	2	200
Atracurium	1	10
Cisatracurium	2	20
Mivacurium	0.2	2

Data from Mertes PM et al. J Invest Allergol Clin Immunol 2011;21:442

[a]Concentrations normally nonreactive in subjects not allergic to a neuromuscular blocking drug

[b]A positive test is a wheal after 20 min with a diameter 3 mm greater than the negative control or a diameter at least half the diameter of the positive control

[c]0.02–0.05 ml injected to give a 4 mm diameter bleb. A positive test is the appearance of an erythematous wheal (often pruritic) after 20 min with a diameter at least twice that of the initial bleb

Positive control for prick test: Histamine 10 mg/ml or codeine phosphate 9 % w/v. Negative control for prick and intradermal tests: Same volume of solvent used for drugs

[d]A high proportion of positive reactions in normal controls has led to suggestions that these prick test concentrations are too high (see text)

demonstration of positive skin prick test responses to these concentrations in approximately half of 30 healthy, non-atopic, anesthesia-naïve male and female volunteers. This result appears to add weight to the earlier report of positive cutaneous reactions without mast cell degranulation in almost all of 30 normal volunteers following intradermal injection of a 1:100 stock solution (100 μg/ml) of rocuronium. This report led to a change in the recommended intradermal test concentration for rocuronium from 100 to 50 μg/ml.

7.4.3.3.2 Comparison of Prick and Intradermal Skin Testing in the Diagnosis of Anesthetic Allergy

In a prospective, non-randomized study involving 212 consecutive patients over a 4-year period, prick testing with undiluted drugs was compared to intradermal testing with diluted drug solutions of standard, accepted concentrations. A positive prick test was taken as a wheal of diameter >4 mm while a positive intradermal test was a persistent wheal greater than 8 mm. Results of the comparison are summarized in Table 7.8. Overall, there was 93 % agreement between the paired tests and differences between the tests were not significantly different. Severe reactions accounted for 135 of the patients and there were 29 patients with minor reactions. Of the 135 severe reactors, the majority (93 patients, 69 %) reacted to NMBDs. In this group of severe reactors, intradermal tests distinguished more positive reactors, but this finding would have been reversed if a prick test wheal size of <4 mm had been selected as the positive cutoff. It should be pointed out that some practitioners of skin testing consider that any wheal should be considered positive for prick testing. Employment of both tests produced more clear diagnostic outcomes than either test alone and it is likely that the safety of subsequent anesthesia would be improved by performing both tests. When doubt exists over the result of either test, both should be carried out. This study provides an interesting and valuable comparison of the diagnostic performances of the two different skin test methods but it should be remembered that the patient numbers were heavily slanted to NMBD reactors. The results therefore, should not be assumed to be immediately applicable to other agents used in anesthesia or, indeed, to other drugs in general.

7.4.3.3.3 Cross-Sensitivity Between Neuromuscular Blocking Drugs

Skin testing with NMBDs is undertaken not only to establish whether or not a reaction during anesthesia was provoked by the drug administered and, at the same time whether the reaction was immune mediated or due to direct mediator release, but also to investigate cross-sensitivity between different NMBDs with the view of identifying not only the causative drug, but also any other NMBDs that do not cross-react in the skin and which may therefore be possible alternative drugs for safe future use. Skin testing for this purpose must therefore include all other commercially available NMBDs. Cross-reactivities of NMBDs detected in skin tests, unlike cross-reactions demonstrated by serum IgE antibodies, are regarded as the most relevant indication of a patient's in vivo recognition and likely response to individual NMBDs. Despite the sometimes apparently clinically non-applicable nature of information on NMBD cross-reactivities derived from antibody investigations, better fine-structural definitions of individual NMBD allergenic determinants combined with skin test results are likely to provide a better basis for interpreting cross-reactivity data and thereby increase the predictability of potentially unsafe reactions to NMBDs and the identification of NMBDs safe for administration (see Sect. 7.4.4 for an extended discussion).

As for skin testing in general, the concentrations used to identify cross-reacting drugs (Table 7.7) are important but with NMBDs the added complication of possible cross-reactions caused by the histamine-releasing properties of drugs such as atracurium and mivacurium must be taken into account. Some guidelines recommend that the skin prick test can be used to detect cross-reactions between NMBDs if the causative drug produces a positive prick test reaction but if the drug is only positive in the intradermal test, then all NMBDs must be tested intradermally. Cross-reactions are more likely between NMBDs

Table 7.8 Comparison of results of prick and intradermal tests in 212 consecutive patients in an anesthetic allergy clinic

Group	No. of patients tested	No. of comparisons	Drug responsible detected by					Agreement between tests	
			Prick	Intradermal	Both	Either	Neither	Agree (%)	Disagree (%)
Minor reactions	29	117	8	5	4	9	20	113 (97 %)	4 (3 %)
Severe reactions	135	965	108	112	104	116	19	890 (92 %)	75 (8 %)
Preoperative sample	13	82	1	1	0	2	11	75 (90 %)	7 (9 %)
Not anaphylactic	35	228	0	0	0	0	35	216 (95 %)	12 (5 %)
Total	212	1,392	117	118	108	127	85	1,294 (93 %)	98 (7 %)

Drugs tested: neuromuscular blocking drugs, induction agents, opioids, colloids, antibiotics, protamine, neostigmine
From Fisher MM, Bowey CJ. Intradermal compared with prick testing in the diagnosis of anaesthetic allergy. Br J Aneasth. 1997;79:59. Reprinted with permission from Oxford University Press

of similar structure such as the benzylisoquinoliniums but again, a detailed understanding of the IgE antibody-binding determinant structures at the fine structural level is needed to put the subject of clinical cross-sensitivity between NMBDs on a better scientific base.

7.4.3.4 Detection of IgE Antibodies Reactive with Neuromuscular Blocking Drugs

7.4.3.4.1 Detection with Neuromuscular Blocking Drugs and Analogs

Following the development and application of the initial immunoassays to detect IgE antibodies to alcuronium, immunoassays designed to detect IgE to other NMBDs were soon developed and used alongside skin tests to diagnose allergic reactions to the range of NMBDs then in use. Concurrently, assays for the detection of *d*-tubocurarine, succinylcholine and gallamine were developed and used to examine, at the molecular level, the extent of cross-reactivity and quantitative relationships for the recognition of different drugs by IgE antibodies in individual sera. For each different drug assay the methodology was essentially the same as that used for alcuronium. The drug, or an appropriate selected analog, was covalently coupled to the solid phase via a spacer arm to form a chemical complex without first linking the drug/analog to a carrier protein. Patients' sera were added to small quantities of the drug complex and any specifically bound IgE antibodies were detected by the addition of a radiolabeled anti-human IgE antiserum. For NMBDs like alcuronium and *d*-tubocurarine, direct chemical coupling of the drug to the insoluble carrier was achieved but with NMBDs such as succinylcholine, decamethonium and gallamine, suitable functional groups for coupling are not present making the formation of drug-solid phases or drug–protein covalent complexes a difficult task without fairly lengthy and complex chemical manipulations. This problem was overcome by utilizing structural analogs with the same terminal allergenic groups as the NMBDs and which could be easily chemically coupled to a solid phase carrier. Succinylcholine can be thought of as two molecules of choline linked by a carbon chain derived from succinic acid and likewise decamethonium has a choline-like structure at each end of the molecule linked by a six-carbon chain (Fig. 7.5). The strategy pursued was therefore to covalently link choline via its available hydroxyl group to the solid phase by bis-oxirane coupling to produce a complex mimicking the terminal groups of succinylcholine and decamethonium. It was reasoned that such a complex should be antigenically similar, if not identical, to succinylcholine- and decamethonium-solid phase complexes. The same strategy was used to prepare a complex to detect IgE antibodies to gallamine. Gallamine's three attached quaternary ammonium groups can be viewed as three molecules of triethylcholine attached as antennae to an aromatic ring. The ethyl analog of choline was therefore synthesized and coupled directly to the solid support to produce a complex suitable for the detection of gallamine-reactive IgE antibodies. Figure 7.6 shows typical

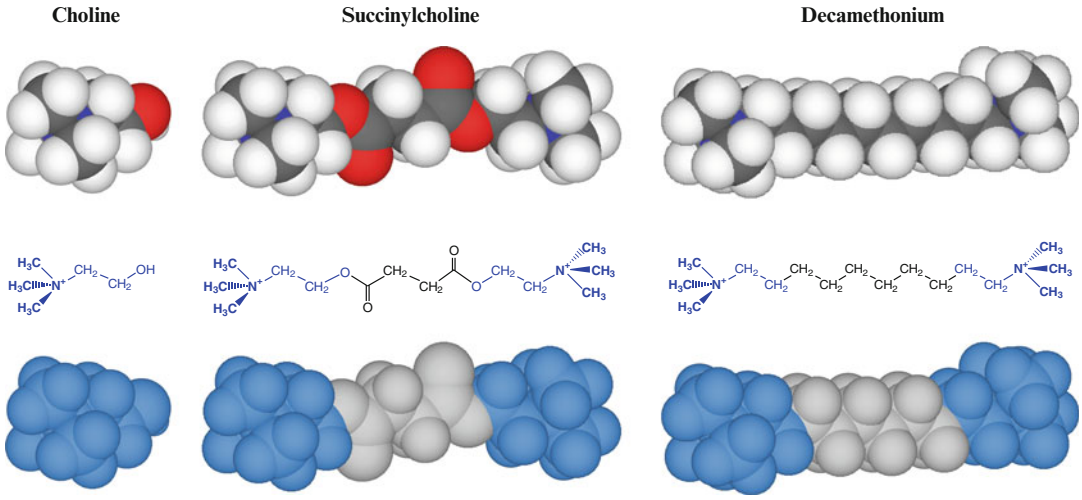


Fig. 7.5 Example of a strategy to overcome the difficulty of preparing solid phase complexes of drugs lacking a suitable functional group for chemical coupling. An example of utilizing structural analogs with the same terminal allergenic groups as the NMBDs to prepare drug conjugates for use in assays to detect NMBD-reactive IgE antibodies. Succinylcholine can be thought of as two molecules of choline (shown in *blue* in the 2D structures and CPK models) linked by a carbon chain derived from succinic acid and likewise decamethonium has a choline-like structure at each end of the molecule linked by a six-carbon chain. Choline was linked via its available hydroxyl group to the solid phase by *bis*-oxirane coupling to produce a complex mimicking the terminal groups of succinylcholine and decamethonium. Such a complex is antigenically similar, if not identical, to succinylcholine- and decamethonium-solid phase complexes

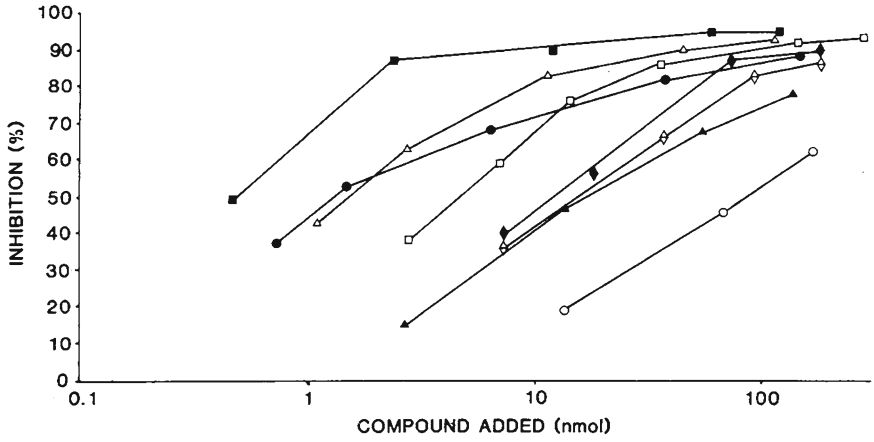


Fig. 7.6 Employment of choline-Sepharose-solid phase conjugate to detect IgE antibodies to the NMBD succinylcholine and other NMBD-reactive IgE in sera of patients allergic to NMBDs. Inhibition of IgE antibody binding to the solid phase by NMBD, choline, and triethylcholine. Key: alcuronium (*open circle*); *d*-tubocurarine (*filled circle*); succinylcholine (*open square*); decamethonium (*filled square*); gallamine (*open triangle*); pancuronium (*filled triangle*); triethylcholine (*open diamond*); choline (*filled diamond*). From Harle DG et al. Assays for, and cross-reactivities of, IgE antibodies to the muscle relaxants gallamine, decamethonium and succinylcholine (suxamethonium). J Immunol Methods 1985;78:293. Reprinted with kind permission from Elsevier Limited

concentration-dependent inhibition profiles obtained when the choline-solid phase is used with serum from a succinylcholine-allergic patient, NMBDs and choline. Typically, cross-reactivity between NMBDs is apparent and although succinylcholine is a good inhibitor, decamethonium, *d*-tubocurarine and gallamine show superior inhibitory potencies and pancuronium and alcuronium are relatively poor inhibitors. These results indicate that detection of IgE antibody combining sites specific for ammonium ion determinants with attached "small" methyl or ethyl alkyl groups. These groups occur on decamethonium, *d*-tubocurarine, gallamine, and succinylcholine, but the ammonium ion determinants on pancuronium, and especially alcuronium, are markedly different in structure and therefore poorly cross-reactive with the former NMBDs. The clear, but inferior to NMBD, inhibition demonstrated by choline is probably related to its single quaternary ammonium group compared to two groups on decamethonium, succinylcholine, and *d*-tubocurarine and three on gallamine. The excellent inhibition shown by gallamine supports this interpretation. For some years, and since the introduction of this choline-Sepharose support for detecting NMBD-reactive antibodies, most tests to detect IgE to succinylcholine have been undertaken with what have been described and reported, especially in the French literature, as "new" solid phases consisting of choline coupled to Sepharose via an ether linkage or coupled *p*-aminophenylphosphorylcholine rather than the originally reported epoxy-coupled solid phase. Apart from offering no conceptual advancement and, in our hands, no significant advantages in performance, these tests can hardly be described as "new."

Brief early attempts to prepare drug-solid phases for the detection of IgE antibodies to the competitive, non-depolarizing, bulky and structurally rigid aminosteroid NMBDs pancuronium, vecuronium and rocuronium, met with only partial success but with the increasing popularity and use of rocuronium in recent years, a Phadia rocuronium ImmunoCap® has been prepared by reacting the drug with succinic anhydride to prepare the hemisuccinate followed by activation to the *N*-hydroxysuccininide ester and final attachment to the solid phase after linkage to a carrier molecule. Recent employment of the ImmunoCap®-based test has produced encouraging results. An ImmunoCap® (Phadia) test for the detection of IgE antibodies to succinylcholine is also available.

The specificity of assays for the detection of NMBD-reactive IgE antibodies in patients' sera generally exceeds 90 % but the specificity may vary from around 50 % reaching a maximum of about 90 %. In one study of assay performance of radioimmunoassays for the detection of IgE antibodies to NMBDs a value of 100 % was obtained for the positive predictive value of a positive result. Skin tests remain the current reference test for NMBD-allergic sensitivity but their specificity is occasionally in question and their sensitivity sometimes less than expected. In addition, skin tests are said to be unreliable in the first 4–6 weeks after the reaction to an NMBD and certain medications may preclude their application in some patients. In these cases and in situations where skin tests cannot be employed, for example in attempts to assess a patient's allergic status before a completed operation or after a fatal reaction, IgE antibody tests on serum from blood samples taken at the time offer the only chance of obtaining the relevant diagnostic data.

7.4.3.4.2 The Use of Morphine for the Detection of IgE Antibodies to Neuromuscular Blocking Drugs

In the original study where IgE antibodies to NMBDs were first detected, morphine was found to potently inhibit IgE antibody binding to the NMBD-solid phase via antibody recognition of its tertiary methylamino group (Fig. 7.2). Subsequent investigations revealed that morphine reacts readily with sera from patients allergic to NMBDs and in comparative inhibition experiments its potency often exceeds that of all NMBDs including the drug that induced the anaphylactic reaction. This indicates that morphine might more closely complement the combining sites of the NMBD-reactive IgE antibodies than the NMBDs themselves. Along with morphine, *d*-tubocurarine also reacts readily and strongly with many sera from NMBD-allergic patients and the two drugs are often the most strongly recognized compounds

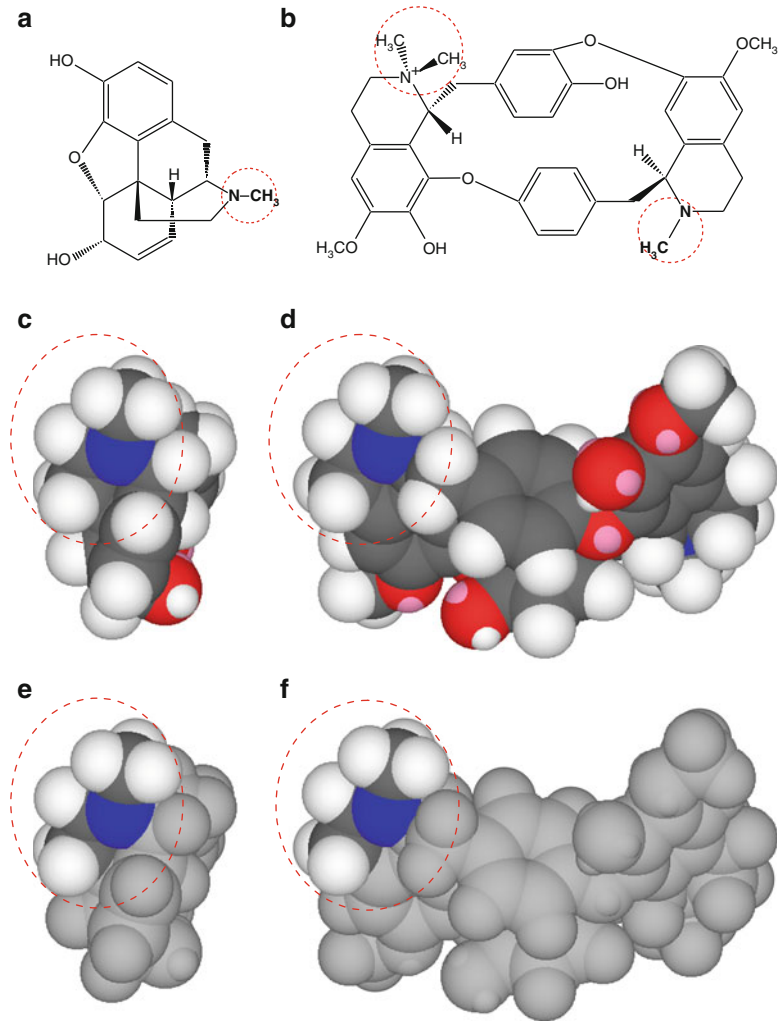


Fig. 7.7 Two-dimensional and CPK space-filling models of morphine (**a**, **c**, **e**) and *d*-tubocurarine (**b**, **d**, **f**). The *circled groups* show the striking similarity of the shapes and orientation of the tertiary *N*-methylamino groups on each compound. This similarity is reflected in the equal reactivity of morphine and *d*-tubocurarine with NMBD-reactive IgE in sera from NMBD-allergic patients (see Harle et al. Mol Immunol 1990: 27:1039). From Baldo BA et al. On the origin and specificity of antibodies to neuromuscular blocking (muscle relaxant) drugs: an immunochemical perspective. Clin Exp Allergy 2009;39:325. Reprinted with permission from John Wiley and Sons

of all the drugs and other chemicals so far examined. The almost equal capacities of morphine and *d*-tubocurarine for interaction with NMBD-reactive IgE antibodies from patients following anaphylaxis to an NMBD prompted further side-by-side comparisons of the drugs including the construction of Corey, Pauling, Koltun (CPK) models. The models (Fig. 7.7) revealed an almost exact likeness in the conformations of atoms of the substituted ammonium groups on one face of each molecule. This very close structural similarity in the antibody-binding regions of the two drugs accounts for their almost equal performance in direct antibody-binding and inhibition tests, but, in addition, both morphine and *d*-tubocurarine exhibit pronounced histamine-releasing properties and the near-identical structural grouping of atoms identified on both drugs may be complementary to a mast cell receptor site involved in direct histamine release by the drugs.

Table 7.9 Incidences of positive IgE antibody test for neuromuscular blocking drugs in the sera of a homogeneous group of patents[a] who experienced an anaphylactic reaction during anesthesia. Comparison of the morphine immunoassay with immunoassays for individual drugs

		Number detected positive for IgE antibodies with	
Patients skin test positive to	Number positive	Morphine test[b]	Neuromuscular blocking drug (or analog) test[c]
One or more neuromuscular blocking drugs	118	100 (85 %)	56/108 (52 %)
Succinylcholine	69	67 (97 %)	47 (68 %)
Rocuronium	15	15	3/13
Vecuronium	10	9	2
Pancuronium	2	0	0
Atracurium	12	3	2/6
Alcuronium	8	4	2
Gallamine	1	1	0
Mivacurium	1	1	0

[a]Patients (118) defined by a positive skin test to one or more neuromuscular blocking drugs and an elevated mast cell tryptase test result
[b]Data from Fisher MM, Baldo BA. Anaesth Intensive Care. 2000;28:167
[c]Tests employing individual neuromuscular blocking drug or analog solid phases

Because of morphine's striking capacity to detect and cross-react with NMBD-reactive antibodies in patients' sera, it was used in solid phase form over a number of years side by side with other immunoassays to detect NMBD-reactive IgE antibodies. This culminated in a study of 347 patients with suspected anaphylaxis to an NMBD where the morphine-solid phase proved a better test for the detection of the antibodies than the other test materials prepared from individual NMBDs or analogs. In an homogeneous group of 118 patients distinguished by an elevated tryptase level and a positive skin test to an NMBD, the morphine immunoassay detected NMBD-reactive IgE antibodies in 100 (84.7 %) of the subjects with a specificity of 98 % and a positive predictive value of 96 %. Efficiency of the assay, that is, the percentage of all results that are true results, was calculated to be 94 %. The morphine test's figures for the detection of IgE to individual NMBDs were 67 out of 69 (97 %) for succinylcholine, 9 out of 10 for vecuronium and 15 out of 15 for rocuronium but only 3 out of 12 for atracurium (Table 7.9). In the assay for succinylcholine-reactive antibodies (using a choline-solid phase), 47 of the 69 sera (68 %) were positive. These results led to the conclusion that the morphine test was an improvement on the battery of individual immunoassays used at that time to aid the diagnosis of allergic reactions to an NMBD and recently this conclusion has been endorsed by the commercial release of the so-called QAM ImmunoCAP® (Thermo Scientific), a form of immobilized morphine designed for use in a fluorescent enzyme immunoassay. Assessment of this test on 168 patients in two hospitals revealed a sensitivity of 84.2 %, almost exactly the same as the sensitivity figure obtained in the original study and the finding that positive reactions to IgE antibodies were significantly higher in skin test-negative reactors (24.6 %) than in controls (9.3 %) suggested that some allergic reactors to NMBDs can be identified by tryptase and serum IgE antibody measurements in some skin test negative patients. It was concluded that the simplicity of the commercial morphine-solid phase assay and its suitability for routine laboratory use made it a valuable addition to skin testing in diagnosing NMBD-allergic sensitivity.

7.4.3.5 Basophil Activation Test

When used as a diagnostic aid for allergy to NMBDs, the basophil activation test (BAT) has been found to be specific but disappointingly lacking in sensitivity. Results for specificity have generally been above 90 %, and although a figure

of 92 % was obtained for sensitivity in one study on rocuronium-induced anaphylaxis, findings have been as low as 36 % and <60 % in five separate studies. Various suggestions to explain the often poor and variable sensitivity results have been advanced and include arbitrarily chosen decision thresholds often based on experience with protein allergens rather than drugs, the failure to include well-characterized control subjects, the possible interference of other medications and the concentrations of NMBDs used. Another contributing factor might be the time elapsed between patient reactions and the performance of the test although NMBD-reactive IgE antibodies are known to be long lasting in some patients (see Sect. 7.4.3.1). In one study the sensitivity increased from a low of 36.1–47.6 % for reactions occurring in ten patients 4–8 years before testing and up to 85.7 % for reactions in six patients within the last 3 years. However, the question does not, as yet, seem to have been systematically investigated and longitudinal studies to determine optimal time intervals are needed. Application of the BAT to aid the management of anaphylaxis to rocuronium led to the conclusion that the method is a reliable diagnostic aid and superior to serum IgE antibody inhibition assays in complementing skin tests for the identification of clinically relevant cross-reactions between NMBDs. Although the BAT can be said to reflect the in vivo pathways leading to allergen-induced mediator release and the resultant allergic manifestations, there seems to be no compelling argument at present for it to replace or add to skin testing and tests for serum IgE antibodies in drug-induced allergy diagnosis. It should also be remembered that the BAT and the skin test are not directly comparable. Even though both tests proceed via FcεRI receptors on the surfaces of basophils and mast cells, the cells involved are different, they are at different stages of differentiation and mast cells at different anatomical sites show significant heterogeneity. There are, however, occasions when an allergic reaction to an NMBD can be identified by clearly positive tryptase and IgE antibody tests despite a negative skin test and in this situation the BAT may be useful to confirm the diagnosis and identify a safe alternative NMBD.

7.4.3.6 Leukocyte Histamine Release Tests

Although not widely used, in vitro leukocyte histamine release tests have been applied with some encouraging results to the diagnosis of allergy to NMBDs. Histamine release was demonstrated in 8 of 25 (32 %) and 26 of 40 (65 %; maximum release 43.8 ± 23.3 %, spontaneous release 1.7 ± 1.1 %) patients with anaphylaxis to an NMBD. The specificity was not determined in the former study but in the latter investigation no histamine release was demonstrated from the leukocytes of 44 control subjects. The application of drug-induced histamine release in drug allergy diagnosis is discussed further in Sect. 4.5.2.

7.4.3.7 Best Current Combination of Tests for the Diagnosis of Immediate Hypersensitivity to Neuromuscular Blocking Drugs

For the diagnostic investigation of an adverse reaction to an NMBD, a reaction that all too often manifests as a life-threatening response with anaphylactic-like symptoms, tests with the capacity to identify the culprit drug and identify any cross-sensitivity with other NMBDs are required. To confirm the release of inflammatory mediators from mast cells, the serum tryptase assay, which measures the concentration of the released enzyme, is currently the best available relevant test. Note that assays for total tryptase released from mast cells and mature tryptase which is a direct measure of mast cell activation now exist and care should be taken to distinguish the two when requesting diagnostic tests, interpreting tryptase concentrations in results provided and when reviewing the literature (see Sect. 4.5.1.3). Although mast cell tryptase is said to be elevated in some anaphylactoid or nonimmune-mediated reactions (eg. red man syndrome following vancomycin), evidence for this is conflicting and elevated levels of the enzyme in patients' sera show strong correlation with the involvement of NMBD-reactive IgE antibodies in reactions to these drugs during anesthesia. In fact, guidelines issued by some professional bodies regard the employment of the tryptase test as "mandatory" in the diagnostic protocol for

drug-induced suspected allergic reactions. Skin testing with NMBDs is the reference test and central to diagnosis such that if only one test were to be used, it would be selected. However, skin testing with NMBDs is not problem- or criticism-free with its sensitivity for some drugs sometimes lacking and its specificity occasionally controversial. In addition to positive responses obtained in cases involving an IgE-dependent mechanism, positive skin tests may occur as a result of nonspecific histamine release. False-positive results obtained with the known histamine releaser atracurium illustrate this point. Importantly, skin tests are also unreliable in the first 4–6 weeks after a reaction to an anesthetic agent and in patients receiving certain medications. Direct binding and inhibition tests for the detection of IgE antibodies that react with NMBDs are valuable adjuncts to skin tests and the tryptase assay. The value of the IgE test is most obvious in cases where clinical data indicates an allergic reaction but skin tests are negative or equivocal and when, unlike skin testing, it can be used in the 4–6 week period immediately following a patient's reaction. Other important applications are tests on preoperative serum samples taken from patients who subsequently experienced a reaction during anesthesia and sera taken just before and after death. Because of its apparent capacity to react with IgE antibodies to all of the NMBDs, its sensitivity, simplicity and suitability for routine laboratory use, the morphine immunoassay (Sect. 7.4.3.4.2) is the best single choice for the diagnostic detection of NMBD-reactive IgE antibodies in patients' sera. If available, other "specific" immunoassays for individual NMBDs may also be used. Note, however, if IgE antibody immunoassays are to yield their full potential as a diagnostic aid for NMBD-induced hypersensitivity responses, much greater attention will need to be paid to gaining immunochemical insights for the interpretation of antibody recognition of individual NMBDs as well as the many factors relevant to the design and interpretation of direct binding and inhibition studies (see Sect. 7.4.4). It should also be remembered that in most cases, the detection of morphine-reactive IgE antibodies is not an indication of clinical hypersensitivity to the drug. Type I IgE antibody-mediated hypersensitivity reactions to morphine occur rarely and the reactions of the drug with antibodies in the sera of NMBD-allergic patients are due to antigenic cross-reactivity of the shared tertiary ammonium groups.

In summary, in addition to a meticulously gathered and recorded history of an NMBD-induced reaction and expert clinical assessment of the signs and symptoms of the reaction, and regardless of the particular NMBD implicated, the "best combination" of investigative procedures currently available comprises:

1. The tryptase test carried out on at least one, but ideally two or more, serum samples drawn at optimum time intervals, namely 30 min up to 4–6 h after the onset of symptoms.
2. Skin tests with unconjugated NMBDs presented either percutaneously or intradermally or both. The skin test is central to identifying the culprit drug as well as any cross-reacting NMBD.
3. The morphine-solid phase assay for detecting IgE antibodies that react with any of the NMBDs. This assay may be supplemented with other immunoassays prepared with a specific NMBD (e.g., rocuronium) or an analog such as choline (for succinylcholine). In the right hands, judicious application of quantitative inhibition studies of antibody binding can add valuable information by identifying the fine structural recognition features of the most reactive NMBDs and the NMBDs most likely to cross-react clinically.

Application of this combination of tests offers the best chance of successfully investigating a hypersensitivity reaction to an NMBD during anesthesia, confirming or eliminating the occurrence of a true IgE antibody-mediated reaction and thus gaining insights into the underlying mechanism.

7.4.4 Cross-Reactions Between Neuromuscular Blocking Drugs

7.4.4.1 Reactivity of Patients' Sera with Different Neuromuscular Blocking Drugs

Both skin and IgE antibody tests can be used to detect allergenic cross-reactivity between NMBDs but the frequency of cross-reactions is generally higher when determined by interaction

with antibody than skin testing. The clinical relevance of serum antibody test results has been questioned and this reflects the different underlying processes of the two tests with the in vitro method detecting interaction of the drugs with the antibody combining sites and the cutaneous test detecting the cross-linking of cell-bound IgE by NMBDs and the subsequent release of inflammatory mediators. Even so, it is true that much of the cross-reactivity data from the in vitro inhibition experiments remains poorly understood and interpreted. As emphasized in this chapter, understanding and interpreting immunological recognition and cross-reactivity of NMBDs depends on defining the precise determinant structure on an individual suspected NMBD as well as taking into account a number of other important factors such as the structure of the antibody-reactive solid phase used in the inhibition study and the flexibilities, conformations, and binding affinities of the all the NMBDs examined. If the structures on NMBDs recognized by their complementary antibody combining sites were confined to the tertiary and quaternary ammonium groups one would expect that, regardless of the particular NMBD involved (and assuming equal accessibility for the antibodies), all NMBDs would show approximately equal reactivity. This is clearly not the case as even a quick perusal of the extensive quantitative inhibition findings set out in Table 7.10 shows, but three main, and interrelated, conclusions can be drawn from the 150 inhibition results obtained when six different NMBDs were used with sera from 13 different patients. Firstly, regardless of the drug that caused the patient's reaction and the drug on the solid phase test material, the clear recognition and superior inhibitory potency of decamethonium is apparent. The reasons for this may be related to the relative flexibility of the decamethonium molecule and the chemical composition of its ammonium groups. The substituted ammonium ions on the flexible, straight chain depolarizing NMBDs like decamethonium and succinylcholine (Fig. 7.8) are more accessible to antibody binding than the ammonium ions on the bulky, rigid molecules such as alcuronium and the aminosteroid NMBDs (Figs. 7.8 and 7.9). Decamethonium has trimethylammonium groups at each end of an extremely flexible ten-carbon chain while succinylcholine with the same terminal structures is less flexible due to the presence of two ester groups in the chain. One might therefore expect that in free solution decamethonium would be more accessible to antibody binding and this would be reflected in its superior inhibitory performance. The *N*-methyl groups of decamethonium might also help explain its excellent inhibitory results. When investigated at the fine structural level, the combining sites of many NMBD-reactive IgE antibodies show specificity for the *N*-methyl rather than *N*-alkyl groups with more carbons, and although the higher alkyl substituents are often recognized, the recognition is clearly weaker. The likely recognition preference for *N*-methyl determinants is also revealed in Table 7.10 by the unexpectedly better than expected inhibitory findings with *d*-tubocurarine (which has a quaternary ammonium ion with two methyl groups and a tertiary methylamino group) and by recent results with atracurium. The quaternary allylpyrrolidinium groups on alcuronium and rocuronium, the morpholino group of rocuronium and the *N*-piperidinium groups on vecuronium and pancuronium (Figs. 7.8 and 7.9) would also be less well recognized by antibody combining sites complementary to determinants carrying the *N*-methyl group.

The second main conclusion drawn from the data set out in Table 7.10 is that there is, not unexpectedly, a relationship between the NMBD that induced the anaphylactic reaction, the drug-solid phase used for testing and the most active inhibitors. The most secure results and results most likely to be relevant to productive immunochemical and clinical interpretation are those obtained in inhibition studies where the NMBD that produced the anaphylaxis matches the NMBD attached to the solid phase. The problem, however, in pursuing this analysis and coming to any firm conclusions is a factor that cannot be ignored, viz., the sensitizing agent(s) for most, if not all, patients who reacted to an NMBD is not known. This is the third, and obvious, conclusion drawn from the results shown here and which must be kept in mind when NMBD–IgE antibody interactions are being considered. The conundrum

Table 7.10 Inhibition of IgE antibody binding to different neuromuscular blocking drug or analog solid phases by individual neuromuscular blocking drugs

Patient's[a] serum	Drug reacted to	Drug or analog[b] on solid phase[c]	Amount (nmol/tube) of NMBD to produce 50 % inhibition of uptake of anti-human IgE					
			Alcuronium	*d*-Tubocurarine	Pancuronium	Succinylcholine	Decamethonium	Gallamine
1	*d*-Tubocurarine	*d*-Tubocurarine	39	17	130	195	105	100
		Alcuronium	39	34	130	100	73	105
2	*d*-Tubocurarine	*d*-Tubocurarine	320	90	470	525	1000	1000
		Choline	23	5	4	13	1.1	11
		Triethylcholine	28	28	5	19	2.9	6
3	*d*-Tubocurarine Alcuronium	*d*-Tubocurarine	16	29	11	240	75	19
		Alcuronium	2	6	15	160	59	42
		Vecuronium[d]	1.1	1.8	0.4	1.3	0.9	1.2
4	Alcuronium	Choline	42	12	9.4	4.1	4	8.7
5	Alcuronium	Choline	30	9	2.9	2.9	1.6	8.7
6	Alcuronium	Choline	15	4.3	10	6.1	2	7.8
		Triethylcholine	15	25	9.4	12	4.3	14
7	Succinylcholine	Choline	41	10	14	7.3	1.3	6.9
8	Succinylcholine	Choline	86	1.3	17	4.7	0.5	1.6
		Triethylcholine	89	0.6	15	6.2	0.4	13
9	Succinylcholine	Choline	3.3	9.7	9.8	11	0.8	9.6
		Triethylcholine	50	74	24	18	3	5.6
10	Succinylcholine	Choline	37	1.8	4.7	6	0.9	6.8
		Triethylcholine	35	5.9	4.9	21	3.2	5.9
		d-Tubocurarine	180	10.1	42.1	52	14	61
11	Succinylcholine Decamethonium	Choline	58	0.7	7.7	9.8	1.9	8.4
		Triethylcholine	68	8	6.7	12	4.5	5.6
		d-Tubocurarine	220	12.5	110	67	46	85
12	Gallamine	Triethylcholine	74	12	14	8.1	7.3	4.2
13	Gallamine	Triethylcholine	750	40	54	55	32	37

From Baldo BA et al. On the origin and specificity of antibodies to neuromuscular blocking (muscle relaxant) drugs: an immunochemical perspective. Clin Exp Allergy 2009;**39**:325). Reprinted with permission from John Wiley and Sons.

[a]Serum from patients who reacted with anaphylaxis after receiving the indicated drug

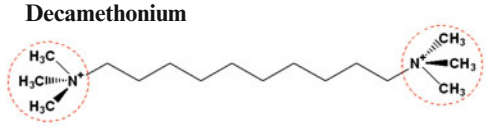
[b]Neuromuscular blocking drug (NMBD) or analog—choline for succinylcholine and decamethonium; triethylcholine for gallamine

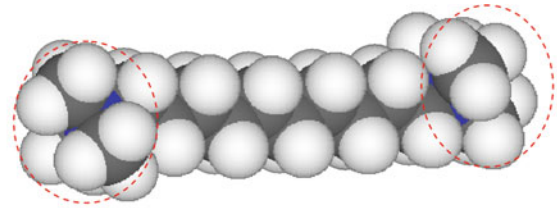
[c]Drug or analog covalently coupled to Sepharose

[d]Vecuronium produced 50 % inhibition at 0.2 nmol/tube

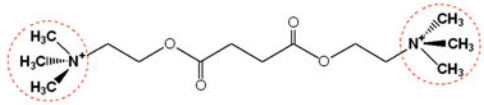
Depolarizing NMBDs–flexible molecules

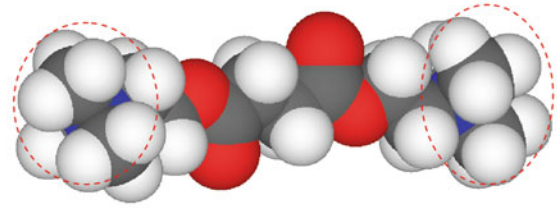
Decamethonium





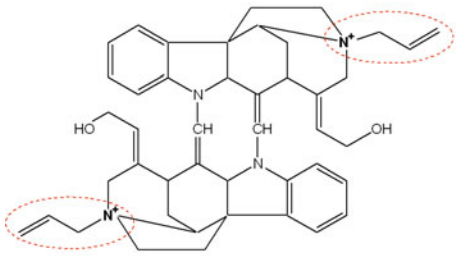
Succinylcholine

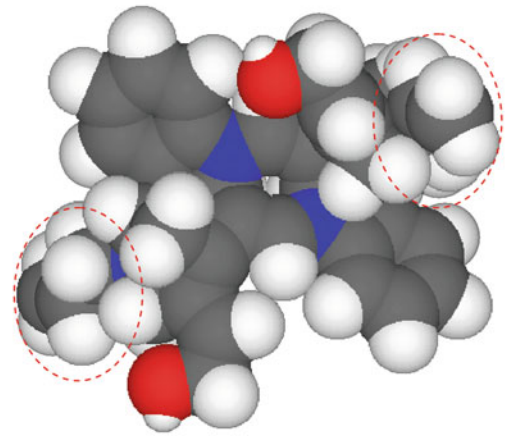




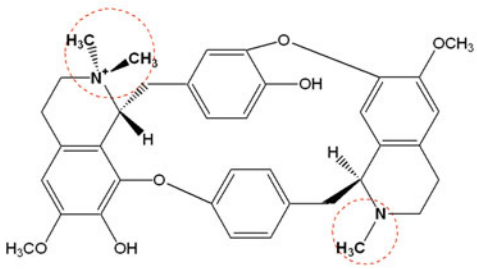
Competitive NMBDs–bulky, rigid molecules

Alcuronium





***d*-Tubocurarine**



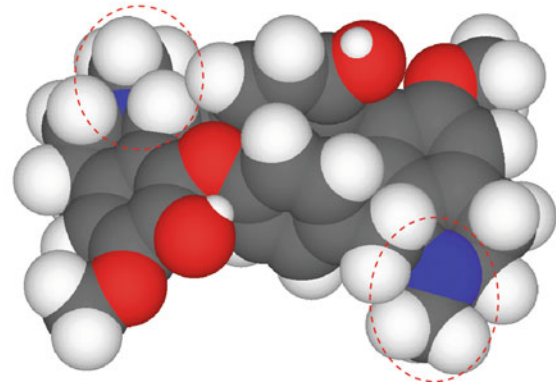


Fig. 7.8 Comparison of two-dimensional structures and space-filling models of straight chain, flexible (decamethonium and succinylcholine) and bulky, rigid (alcuronium and *d*-tubocurarine) NMBDs. Relative accessibilities of substituted ammonium groups on depolarizing and competitive NMBDs. The freehand flexibility of decamethonium is due to two trimethylammonium groups at the ends of a ten-sp^3-carbon chain while flexibility of succinylcholine is somewhat more restricted by the presence of two ester groups containing an sp^2 carbon linked to an sp^2 oxygen. Two relatively large allylammonium groups are not easily accessible in the bulky structure of alcuronium while the quaternary ammonium group, and especially the tertiary methyamino group on *d*-tubocurarine, are overtly exposed. From Baldo BA et al. On the origin and specificity of antibodies to neuromuscular blocking (muscle relaxant) drugs: an immunochemical perspective. Clin Exp Allergy 2009;39:325. Reprinted with permission from John Wiley and Sons

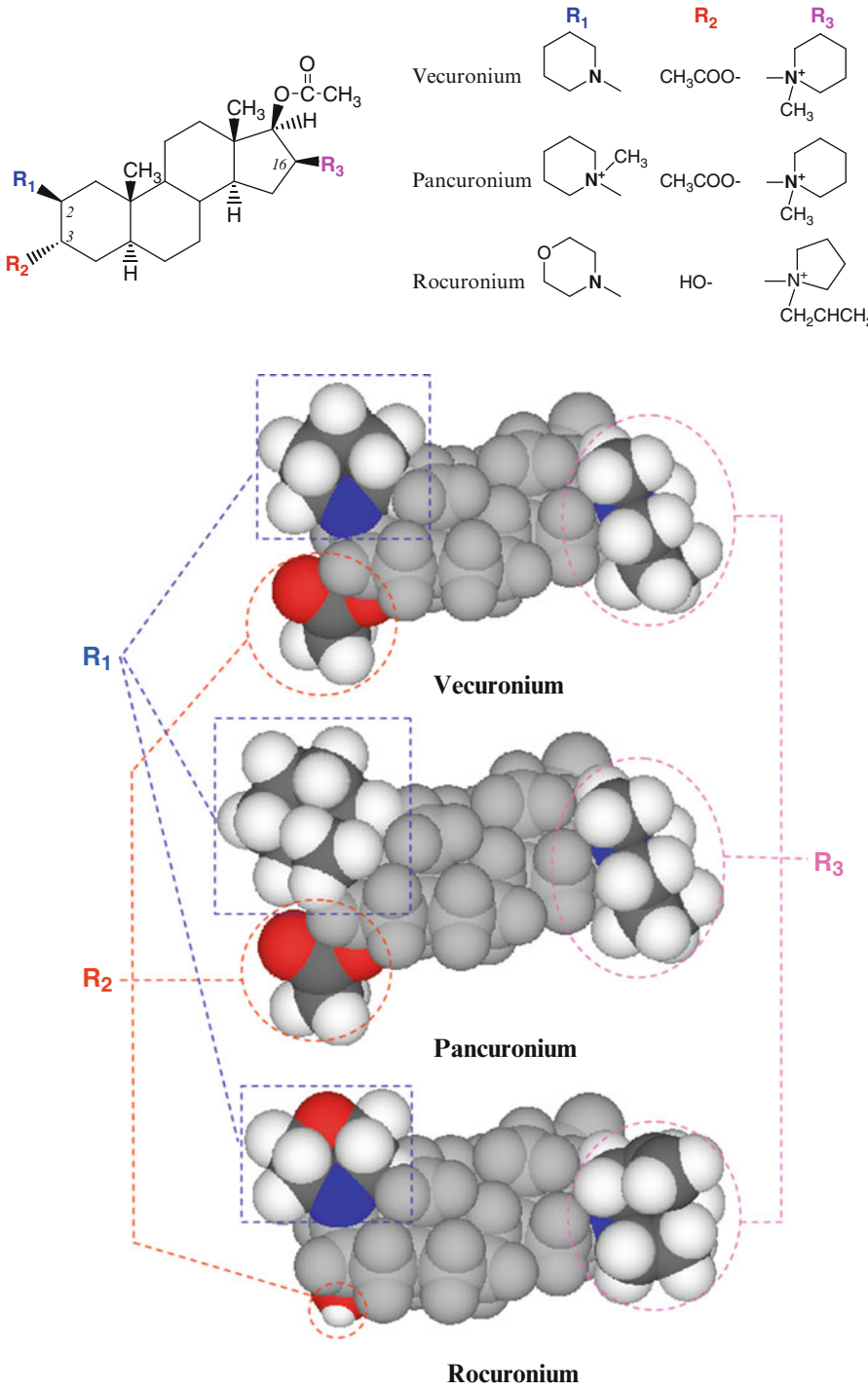


Fig. 7.9 Two-dimensional and space-filling models of the bulky and rigid competitive aminosteroid NMBDs vecuronium, pancuronium, and rocuronium. Note the structural differences of the three compounds at R_1 and at R_2 and R_3 for rocuronium. From Baldo BA et al. On the origin and specificity of antibodies to neuromuscular blocking (muscle relaxant) drugs: an immunochemical perspective. Clin Exp Allergy 2009;39:325. Reprinted with permission from John Wiley and Sons

of the sensitizing agent(s) is examined in more detail below.

7.4.4.2 Cross-Reactions of Neuromuscular Blocking Drugs at the Clinical Level

The frequently used aminosteroid NMBD rocuronium provides a good example of our current understanding of cross-sensitivity of NMBDs from the clinical perspective. Soon after rocuronium's release, its cross-reactivity with five other NMBDs was assessed by skin testing, reactivity with serum IgE antibodies and leukocyte histamine release. Of the 31 patients included in the study, 30 showed some cross-reactivity with at least one NMBD and ten did not cross-react with rocuronium leading to the suggestion that the NMBD might be safe for those patients. In addition, of five patients positive to all the NMBDs examined, one patient showed no cross-reactivity at all with rocuronium. A recent assessment of cross-reactivity between rocuronium and vecuronium, succinylcholine and benzylisoquinoline employing skin tests, serum IgE antibody assays and the BAT found that 41 patients (69 %) with established rocuronium allergy showed evidence of cross-reactivity with vecuronium. For succinylcholine, 76 % of the rocuronium-allergic group had serum IgE antibodies that reacted with the drug but only one-third of these patients showing serum IgE-reactivity had a positive skin test and/or BAT to succinylcholine. Cross-reactivity with the benzylisoquinolines cisatracurium and atracurium was either entirely absent or, at most, equivocal in four patients.

7.4.4.3 A Topical Practical Example of the Value of Insights Obtained from Cross-Reaction Studies: Rocuronium and the Risk of Anaphylaxis

Rocuronium also provides a good example of allergenic cross-reactivity detected by IgE antibodies from the immunological perspective. With rocuronium's increasing popularity and wide usage has come a coincident increase in reports of anaphylaxis to the drug leading some clinicians to call for closer monitoring of the reports and their

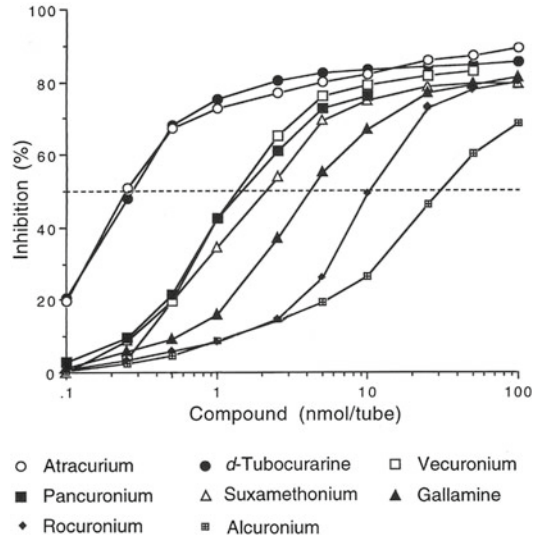


Fig. 7.10 Concentration-dependent inhibition of binding of IgE antibodies specific for tertiary and quaternary methyl (mainly) and ethyl amino groups by neuromuscular blocking drugs. Side-by-side quantitative comparisons of inhibitory potencies of rocuronium and alcuronium with six other neuromuscular blocking drugs. Refer to Table 7.11 for the precise quantitative relationships. The *dashed line* indicates 50 % inhibition. From Pham NH et al. Studies on the mechanism of multiple drug allergies. Structural basis of drug recognition. J Immunoassay Immunochem. 2001;22:47. Reprinted with permission from Taylor & Francis

frequency. Increasing concern about the drug's safety culminated in the Norwegian Medicines Agency publishing an alert and recommending restriction of usage to urgent intubations but others have claimed that false-positive skin tests overestimating the incidence of anaphylactic cases and/or the increased usage and market share are the reasons for rocuronium's alleged risk.

An alternative possible explanation based on a structural perspective has recently been advanced. In an immunochemical investigation, the relative inhibitory potencies of rocuronium and seven other NMBDs were compared side by side in quantitative hapten inhibition experiments using a serum containing IgE antibodies with a precisely defined specificity for tertiary and quaternary "small," that is, methyl and ethyl (ethyl less strongly recognized), mono, di and tri (di and tri less strongly recognized), alkyl amino groups (Fig. 7.10). In keeping

Table 7.11 Demonstration of cross-reactivity between neuromuscular blocking drugs. Results of immunoassay inhibition studies[a] with a patient's serum containing IgE antibodies reactive with "small" ammonium groups[b]

Neuromuscular blocking drug	Alkylamino or alkylammonium group	Structure(s) of group(s)	Amount (nmol/tube) of drug for 50 % inhibition of binding of IgE antibodies
Atracurium	2 Quat methylammonium	$-N^+-CH_3$	0.25
d-Tubocurarine	1 Quat dimethylammonium	$N^+(CH_3)_2$	0.25
	1 Tert methylammonium	NH^+-CH_3	
Vecuronium	1 Quat methylammonium	$-N^+-CH_3$	1.4
	1 Tert amino	$N-H$	
Pancuronium	2 Quat methylammonium	$-N^+-CH_3$	1.5
Succinylcholine	2 Quat trimethylammonium	$-N^+(CH_3)_3$	2
Gallamine	3 Quat triethylammonium	$-N^+(C_2H_5)_3$	4
Rocuronium	1 Quat allylammonium	$-N^+-CH_2-CH=CH_2$	10
	1 Tert amino	$N-H$	
Alcuronium	2 Quat allylammonium	$-N^+-CH_2-CH=CH_2$	30

Data from Pham NH et al. J Immunoassay Immunochem 2001; 22:47
Tert tertiary, *Quat* quaternary
[a]For experimental details see Pham NH et al. J Immunoassay Immunochem 2001;22:47
[b]Tertiary and quaternary mono-, di- and tri-alkylamino groups but only if alkyl groups are "small", viz., methyl or perhaps ethyl

with the recognition specificity of the antibody combining sites and its complementary dimethylamino antigen-solid phase, NMBDs containing methylammonium groups were the most strongly recognized structures. Thus, atracurium and *d*-tubocurarine were the most active inhibitors followed by vecuronium, pancuronium, succinylcholine, and gallamine in that order. Table 7.11 relates the structures of the ammonium ions on the NMBDs to the inhibitory potencies of all eight NMBDs examined. The most poorly recognized structures were rocuronium and alcuronium, requiring 40 and 120 times as much drug respectively as alcuronium and *d*-tubocurarine for 50 % inhibition. These results show clearly that the best recognized structures contain at least one quaternary methyl or dimethylammonium ion of simple structure (atracurium and *d*-tubocurarine) and as these groups are replaced by a tertiary amino group (vecuronium) and quaternary methylammonium ions of greater complexity (the methylpiperidinium structure in vecuronium and pancuronium and trimethylammonium ions of succinylcholine), recognition by antibody declines (Table 7.11). The markedly poorer inhibitory potencies exhibited by rocuronium and alcuronium can be explained by the presence in both drugs of the *N*-allyl (also called propenyl) pyrrolidinium quaternary ammonium ion (Fig. 7.11a, b) and this conclusion is reinforced by the significantly weaker inhibition obtained with alcuronium which has two of these groups compared to rocuronium which has one. A comparison of the structures set out in Fig. 7.11 provides a visual perspective to this structure–activity interpretation revealing the marked differences in size, shape and orientation between the methylammonium group on most NMBDs, for example the methylammonium ion of vecuronium (Fig. 7.11c, e) and the allylammonium ion of rocuronium and alcuronium

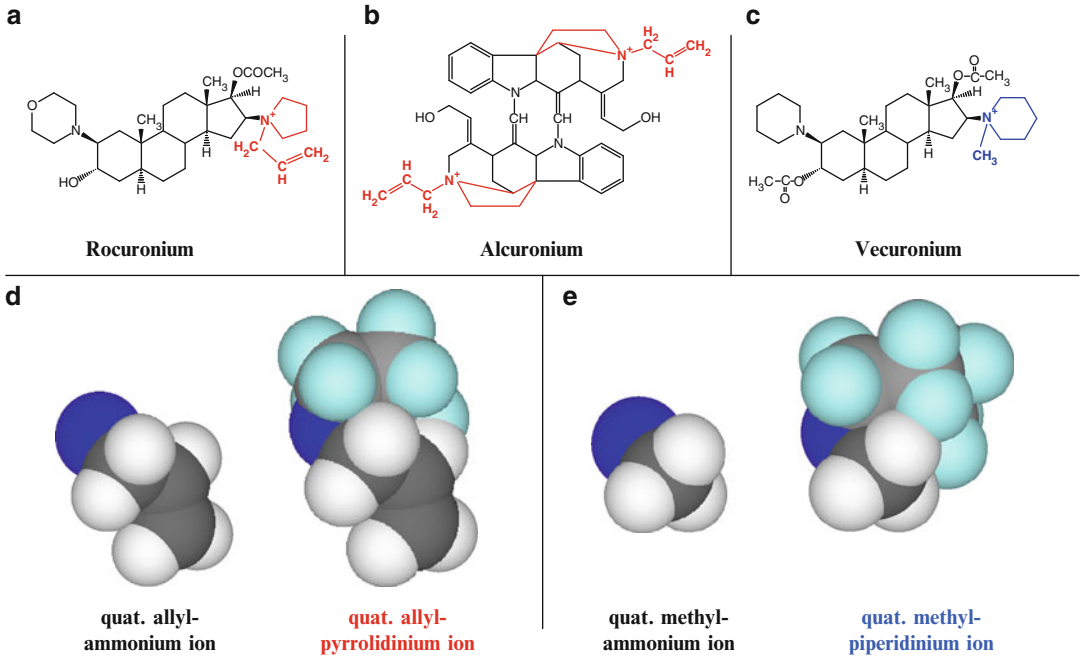


Fig. 7.11 Two-dimensional structures of (**a**) rocuronium, (**b**) alcuronium, and (**c**) vecuronium with the quaternary allylpyrrolidinium ions of rocuronium and alcuronium shown in *red* (**a** and **b**) and the quaternary methylpiperidinium ion of vecuronium shown in *blue* (**c**). Three-dimensional CPK molecular models of the quaternary allylammonium and allylpyrrolidinium ions are shown in (**d**) and the quaternary methylammonium and methylpiperidinium ions are shown in (**e**). In (**d**) and (**e**), hydrogen and carbon atoms of the pyrrolidine and piperidinium rings are colored *light blue* and *gray*, respectively, to distinguish these groups from the allylammonium and methylammonium ions

(Fig. 7.11d). In the late 1970s and early 1980s alcuronium was, like rocuronium now, also said to be more likely to provoke allergic reactions than other NMBDs. It may be, for reasons not understood, that the allylpyrrolidinium group makes these two NMBDs somewhat more anaphylactogenic and therefore a greater risk than other NMBDs. By combining these structural insights with experiments designed to compare IgE antibody-mediated release of mediators (for example, in basophil activation and histamine release studies) it might be possible to implicate or exonerate the allylpyrrolidinium specificity as an added risk for NMBD-induced anaphylaxis.

The above findings demonstrate that as well as viewing and interpreting cross-reactions of NMBDs from the purely clinical perspective provided by a patient's symptomatology, skin tests and the tryptase assay, it remains important to consider both the underlying structural features of the NMBDs involved and the structure–activity relationships demonstrated in quantitative IgE antibody binding and recognition (from inhibition studies) experiments.

7.4.5 The NMBD–IgE Conundrum: The Origin of IgE Antibodies to Neuromuscular Blocking Drugs

7.4.5.1 The Speculations and Evidence so Far

In both clinical and research areas of NMBD hypersensitivity, much of the difficulty in interpreting and explaining results of IgE recognition studies stems from our lack of information on the precise specificity(ies) of the preexisting antibodies found in the sera of patients exhibiting allergic sensitivity to the drugs. These IgE antibodies

are present in the sera of apparently normal, healthy subjects before they are exposed to an NMBD and, as discussed above, appear to occur in a significant proportion of the general population, at least in France and Scandinavia. To be in a position to better understand some of the seemingly anomalous results of direct binding, inhibition and cross-reactivity experiments, knowledge of the antigenic source of the NMBD-reactive antibodies is essential. The answer to a second intriguing and important question—why are the antibodies of the IgE class?—might bring with it clues for the evolution and role of immunoglobulin E in humans and perhaps contribute to a better understanding of the presence of preexisting allergic sensitivities to some drugs and other allergens and even a role for IgE in the pathogenesis of some infectious diseases. In seeking answers to these two questions, the most obvious existing clue seems to be the substituted ammonium (tertiary and quaternary) groups present in all the NMBDs and complementary, at least in part, to the NMBD-reactive IgE antibodies. An early suggestion in 1983 of the origin of the antibodies, viz. stimulation by environmental agents in the form of cosmetics, hair products, household chemicals and toilet items etc remains appealing and appears to explain a number of aspects of sensitization but little or no substantive evidence has been forthcoming and, more recently, other speculative explanations involving mammalian rhesus (Rh) ammonium transport proteins and phosphorylcholine antigens (see Sect. 7.4.5.3) have been advanced. Again, these two suggestions remain unsupported by evidence but both are amenable to experimental investigation. A new theory with some evidence has, however, recently been advanced from Scandinavia and this will be outlined in the following section.

7.4.5.2 Pholcodine and Anaphylaxis to Neuromuscular Blocking Drugs in Scandinavia

Starting with the knowledge that anaphylactic reactions to NMBDs are six times more common in Norway than in Sweden, researchers looked for IgE antibodies to succinylcholine and morphine and found positive reactions to the NMBD in 0.4 % and 3.7 % and to morphine in 5 % and 10 % of Norwegian blood donors and allergic subjects, respectively. No serum from Sweden was positive to either drug. When the morphine analog pholcodine, a cough suppressant sold without restriction in Norway but not in Sweden, was tested, sera from 6 % of Norwegian blood donors, but no Swedish sera, reacted positively. In addition, tests for IgE antibodies to pholcodine and morphine on sera from 65 anaphylactic Norwegians showed a similar incidence of positive reactions. This suggested that pholcodine might be the source of sensitization to NMBDs in Norway and this appeared to be supported by a large boost in IgE antibodies to pholcodine, morphine, and succinylcholine following dosage with a cough syrup containing pholcodine. A follow-up study involving the administration of pholcodine to patients with IgE-mediated anaphylaxis to NMBDs again revealed increases in IgE antibody levels, but a boost to total IgE levels was also seen. Maximum serum concentrations of immunoglobulin E were much higher than the sum of the identified antibodies, suggesting at least some non-pholcodine-specific stimulation of IgE. This seems like an important point. Experiments 20 years ago with sera containing high levels of total IgE from subjects with no history of allergy to NMBDs but with eczema, aspergillosis, food allergies, and/or asthma revealed clear reactions of IgE with NMBDs and morphine in direct binding and inhibition experiments and recent tests on 32 subjects with no hypersensitivity to NMBDs but total IgE concentrations in excess of 1,500 kU/l showed positive reactions to pholcodine and morphine in approximately one-third of the subjects. In the latter study, IgE antibodies to morphine and pholcodine were found in 88 and 86 %, respectively, of patients allergic to rocuronium but positive reactions to these drugs were detected in only 0.5–1 % of sera from 95 healthy controls, 95 atopic controls, and nine subjects exposed to morphine.

In morphine, codeine, and pholcodine the determinant structure recognized by NMBD-reactive IgE antibodies, that is, the tertiary *N*-methyl group, is freely accessible so there

seems to be no reason why all three drugs would not react equally well with antibodies. The three compounds differ in structure only at position 3 where morphine has a hydroxyl group, codeine a methoxy, and pholcodine a morpholinylethyl group (Fig. 7.12) so if pholcodine, but not its two analogs, induces allergic sensitization and has an immunological boostering effect on IgE antibody levels, the conclusion seems inescapable, viz., if these effects are real, only the morpholinoethyl group can account for the differences. A possible mechanism for such sensitization remains to be elaborated. In 2009, results of a multinational, multicenter study on national pholcodine consumption and the prevalence of IgE sensitization were published with the conclusions that the result "lends additional support to the PHO (pholcodine) hypothesis" and "that other, yet unknown, substances may lead to IgE-sensitization towards NMBAs." A significant positive association between pholcodine consumption and prevalences of IgE sensitization to pholcodine and morphine but not to succinylcholine or choline hapten (used as mimic for the succinylcholine IgE-binding determinant) was found, but absence of succinylcholine sensitivity in some countries with significant levels of anti-pholcodine IgE antibody raises doubts about the hypothesis.

In February 2011 the European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) began a review of the safety and effectiveness of pholcodine following concerns that the drug might put people at risk from anaphylaxis during anesthesia. At the time of the review, medicines containing pholcodine had been withdrawn from the market in Norway in 2007 (in Sweden this occurred in the 1980s), further publications from Scandinavia had stated that pholcodine use may increase the likelihood of anaphylaxis to NMBDs and the French medicines regulatory agency had asked the CHMP to assess the risk-benefit balance of pholcodine and decide whether or not the marketing authorizations for the drug should be changed. In November 2011 the European Medicines Agency completed its review concluding that the existing evidence against pholcodine is "weak," the drug's "benefits outweigh its risks" and "all marketing authorizations for medicines containing pholcodine should be maintained throughout the European Union."

7.4.5.3 Phosphorylcholine and Neuromuscular Blocking Drug-Reactive IgE Antibodies

Phosphorylcholine occurs widely in natural products, connected via phosphodiester linkages to *N*-acetylglucosamine in proteoglycans and glycolipids. It also occurs in macromolecules that have immunomodulatory and anti-inflammatory properties such as promotion of Th2 responses and inhibition of pro-inflammatory cytokine production by macrophages. "Natural" antibodies, that is, antibodies formed without exposure to foreign antigens via infection or passive or active immunization, also occur widely and such antibodies to phosphorylcholine develop under sterile conditions in mice and are found as nonpathogenic autoantibodies produced by CD5+/B-1 B cells in humans. Some monoclonal antibodies in both the mouse and humans and the acute phase protein, C-reactive protein, have combining sites complementary to phosphorylcholine, the immunodominant structure in "C-substances" a teichoic acid of *Streptococcus pneumoniae*, and a component of other C-substance-like antigens widely distributed in some other bacteria, fungi, protozoa, plants, arthropods, and helminthes. C-substances, isolated by affinity chromatography on C-reactive protein and mouse anti-phosphorylcholine IgA antibodies from house dust mites, intestinal worms, and fungi, especially dermatophytes, were shown to have allergenic properties by skin prick test, IgE antibody binding. and histamine release studies. C-substances precipitate with C-reactive protein and anti-phosphorylcholine monoclonal antibodies, both reactions are inhibited by phosphorylcholine, and there is evidence that anti-phosphorylcholine antibodies have a protective effect against *S. pneumoniae* infections and artherosclerosis. Antigens containing phosphorylcholine are common in helminthes as are anti-phosphorylcholine-specific IgE antibodies in infected hosts, so, together with natural IgE

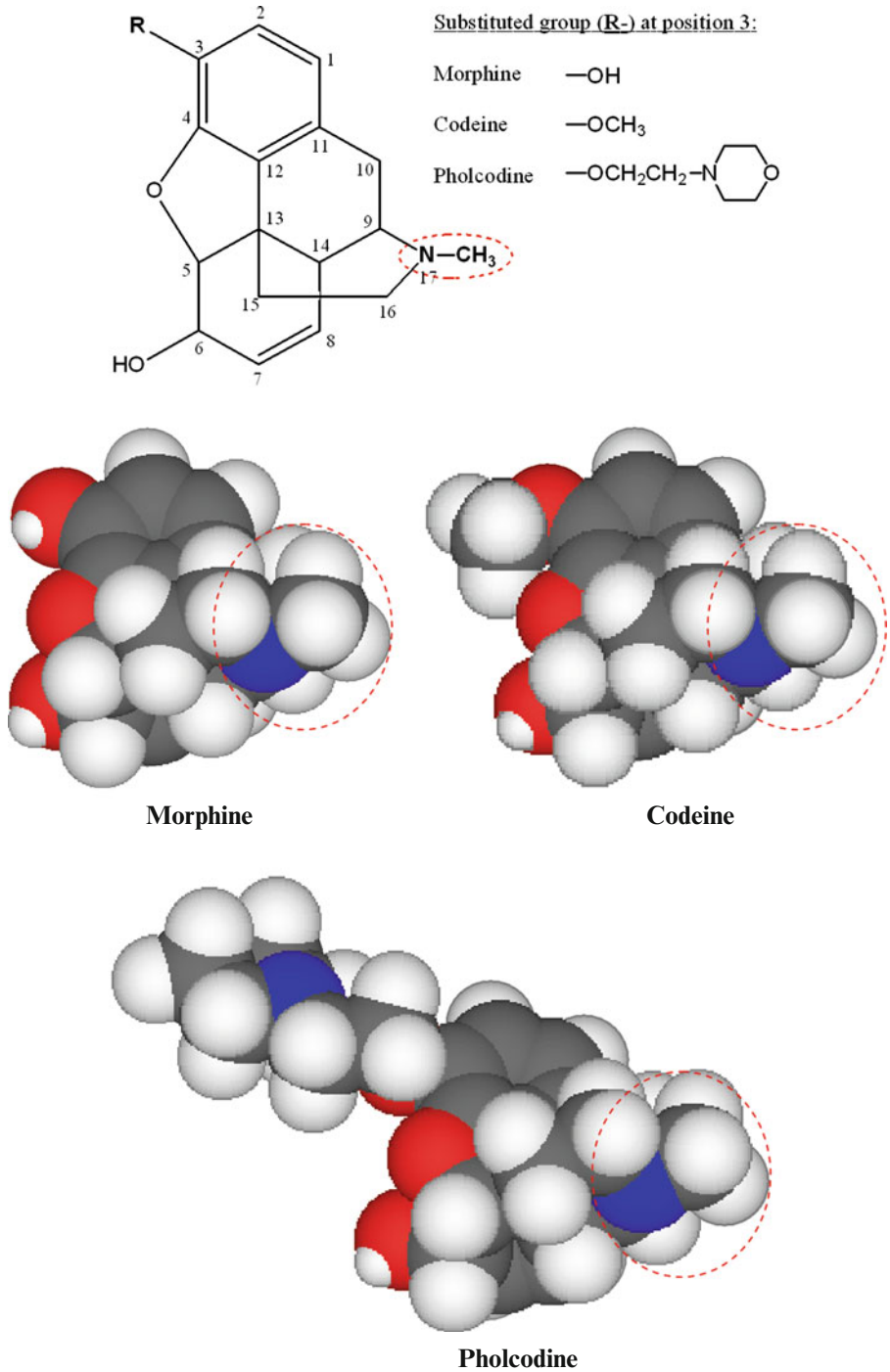


Fig. 7.12 Two-dimensional structure and space-filling models of morphine, codeine, and pholcodine showing the different substituents (R) at position 3. Note that any substituent at position 3 on all three compounds cannot restrict access of antibodies to the *N*-methyl substituent at position 17 on the opposite side of each of the three drugs. From Baldo BA et al. On the origin and specificity of antibodies to neuromuscular blocking (muscle relaxant) drugs: an immunochemical perspective. Clin Exp Allergy 2009;39:325. Reprinted with permission from John Wiley and Sons

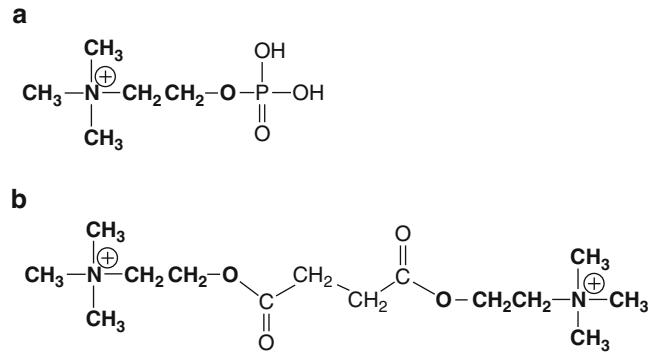


Fig. 7.13 Structural identity (shown in *bold*) of (**a**) the polar head region of the phosphorylcholine molecule and (**b**) both termini of the NMBD, succinylcholine

antibodies to this commonly encountered hapten, phosphorylcholine-reactive immunoglobulins E antibodies are likely to be not uncommon in human populations. In the normal situation, the IgE antibody-binding so-called cross-reactive carbohydrate determinants derived from some plant-derived foods, pollens, and Hymenoptera venoms, and the phosphorylcholine determinant, act like "dormant" allergens demonstrating no clinical relevance as evidenced by absence of clinical symptoms and negative skin tests in patients. However, in both laboratory animals and humans, intravenous challenge with some antigens, for example, parasite glycoconjugates in rats and a galactose disaccharide in humans, provokes an anaphylactic response mediated by preexisting IgE antibodies in the patients' sera. In these cases, the host has gone from a clinically benign or dormant situation where presensitization to an allergen exists, to an unsuspected and severe immediate type I anaphylactic response. It seems likely that a similar response might occur if an antigen containing phosphorylcholine was presented intravenously to subjects with natural or immune anti-phosphorylcholine IgE antibodies. However, with little likelihood of C-substances being administered intravenously and with this potential allergenic determinant usually inaccessible as a component of phosphatidylcholine and sphingomyelin, the conversion of a dormant to an active allergic response mediated by a phosphorylcholine–IgE antibody interaction seems unlikely. The intravenous administration of an NMBD during anesthesia creates, however, the circumstances whereby structures that closely resemble, or are identical to, the quaternary ammonium group of phosphorylcholine, can bind to preexisting IgE antibodies in patients' sera and precipitate an anaphylactic reaction. Figure 7.13 shows the structural identity of both termini of the NMBD succinylcholine and the polar head region of the phosphorylcholine molecule. Cross-reaction between the substituted ammonium groups of succinylcholine and the ammonium groups of other NMBDs means that anti-phosphorylcholine IgE antibodies will also react, to equal or lesser extent, with these NMBDs. Investigations undertaken with this speculation in mind should ultimately reveal its correctness or otherwise.

7.4.6 Sugammadex and Anaphylaxis to Rocuronium

7.4.6.1 Sugammadex and Its Binding to Rocuronium

The naturally occurring γ-cyclodextrin is a circular oligosaccharide made up of eight D-glucopyranoside units in rigid $^4C_1$ conformation and linked α(1-4) around a central cavity (Fig. 7.14a). The oligosaccharide has a truncated cone or toroidal shape with a total of eight primary hydroxyl groups on carbon 6 at the narrow or so-called primary end and 16 secondary hydroxyls on carbons two and three of each glucose unit at the wider or secondary rim of the molecule (Fig. 7.14b). Cyclodextrins are used in the pharmaceutical, food, and cosmetic industries to encapsulate molecules within their hydrophobic cavities, thus forming stable guest-inclusion complexes to improve solubility, stability, and delivery of drugs, prevent drug interactions, and reduce irritation, unpleasant tastes, and smells.

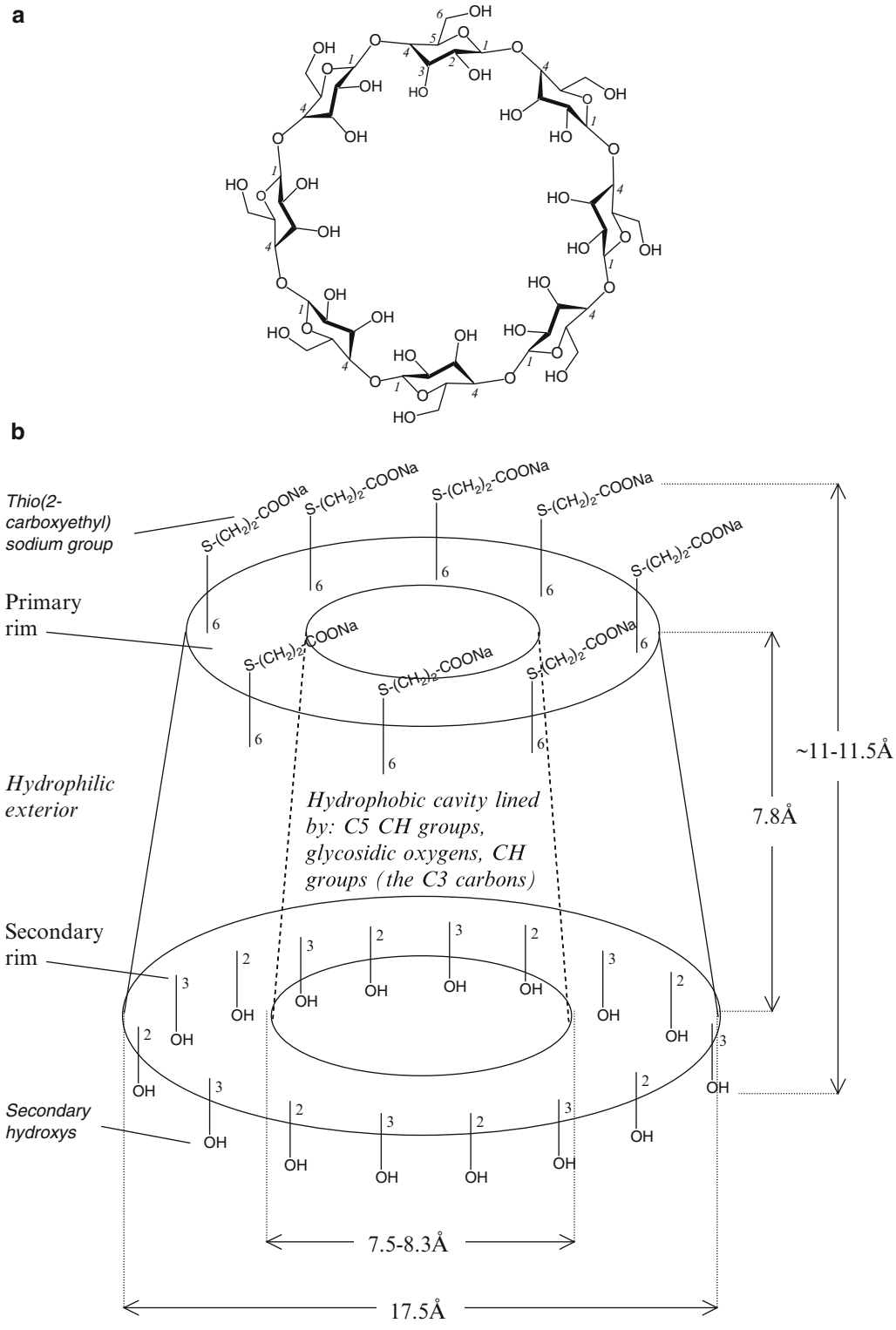


Fig. 7.14 (**a**) Two-dimensional chemical structure of **γ**-cyclodextrin, made up of eight glucopyranose units connected in a circle by **α**-1,4 bonds. (**b**) Diagammatic representation of the toroidal shape of sugammadex showing the attachment of eight thio(2-carboxyethyl)sodium groups at the primary face and indicating the extension of the cavity length by 3.2–3.7 Å (7.8 Å to 11–11.5 Å) by addition of these groups. See also Fig. 7.16. From Baldo BA et al. The cyclodextrin sugammadex and anaphylaxis to rocuronium: Is rocuronium still potentially allergenic in the inclusion complex form? Mini Rev Med Chem 2012;12:701. Reprinted with permission from Bentham Science Publishers

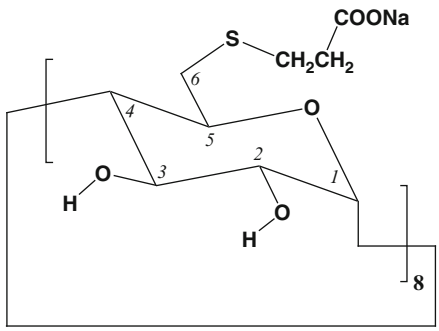


Fig. 7.15 Chemical structure of one of the eight **α**-1-4-linked glucopyranose units of sugammadex, 6-perdeoxy-6-per(2-carboxyethyl)thio-**γ**-cyclodextrin sodium salt, showing the thio(2-carboxyethyl)sodium group linked at position 6 of the glucose molecule. From Baldo BA et al. Drug-specific cyclodextrins with emphasis on sugammadex, the neuromuscular blocker rocuronium and perioperative anaphylaxis: implications for drug allergy. Clin Exp Allergy 2011;41:1663. Reprinted with permission from John Wiley and Sons

For a stable inclusion complex to form, the guest molecule must preferably be fully accommodated by the cyclodextrin and this sometimes involves extension of the cyclodextrin carrier by chemical means. This was the strategy employed to encapsulate and accommodate rocuronium. In an attempt to encompass the entire six rings of rocuronium, chemical modifications were carried out to elongate the cavity by per-6 substitution of each of the primary hydroxyls with propionic acid side chains each linked by a thiol-ether group (Fig. 7.15). This achieved a lengthening of the cyclodextrin carrier from ~7.8 Å to about 11.5 Å, the approximate distance between the C3 carbon of ring A and C16 of ring D on the rocuronium molecule (Fig. 7.16), with the four rings A, B, C, and D within the cavity and the pyrrolidinium quaternary ammonium and the morpholine groups visible at the primary and secondary faces, respectively (Fig. 7.17). A method to encapsulate the NMBD had been sought to aid solubility and

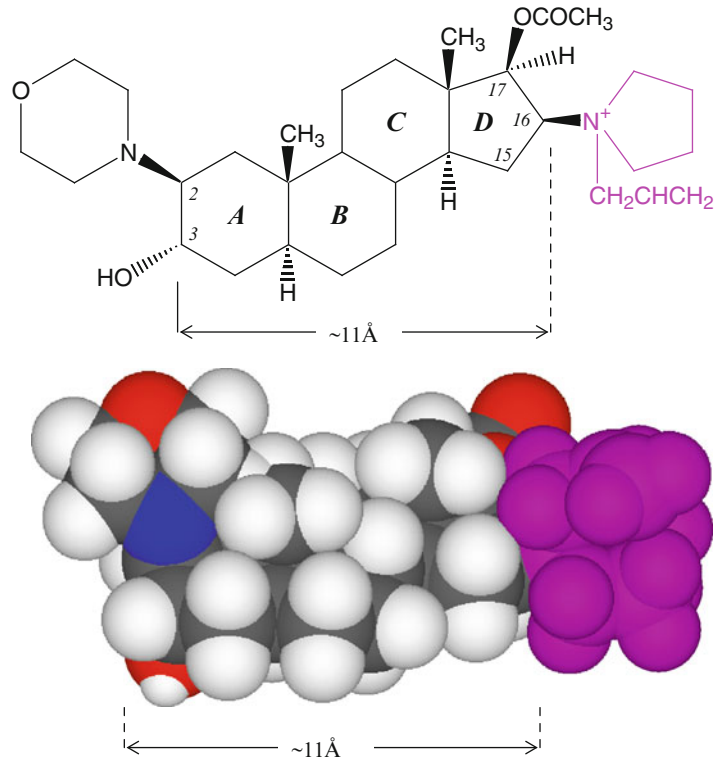


Fig. 7.16 Two-dimensional chemical structure and space-filling CPK molecular model of rocuronium showing the ~11 Å distance between the C3 atom on ring A of the gonane nucleus and the C16 atom on ring D. The propenyl (allyl) pyrrolidinium quaternary ammonium group is shown in *magenta* on the two-dimensional structure and the CPK model. See also Figs. 7.14b and 7.17 for the significance of the ~11 Å distance. From Baldo BA et al. The cyclodextrin sugammadex and anaphylaxis to rocuronium: Is rocuronium still potentially allergenic in the inclusion complex form? Mini Rev Med Chem 2012;12:701. Reprinted with permission from Bentham Science Publishers

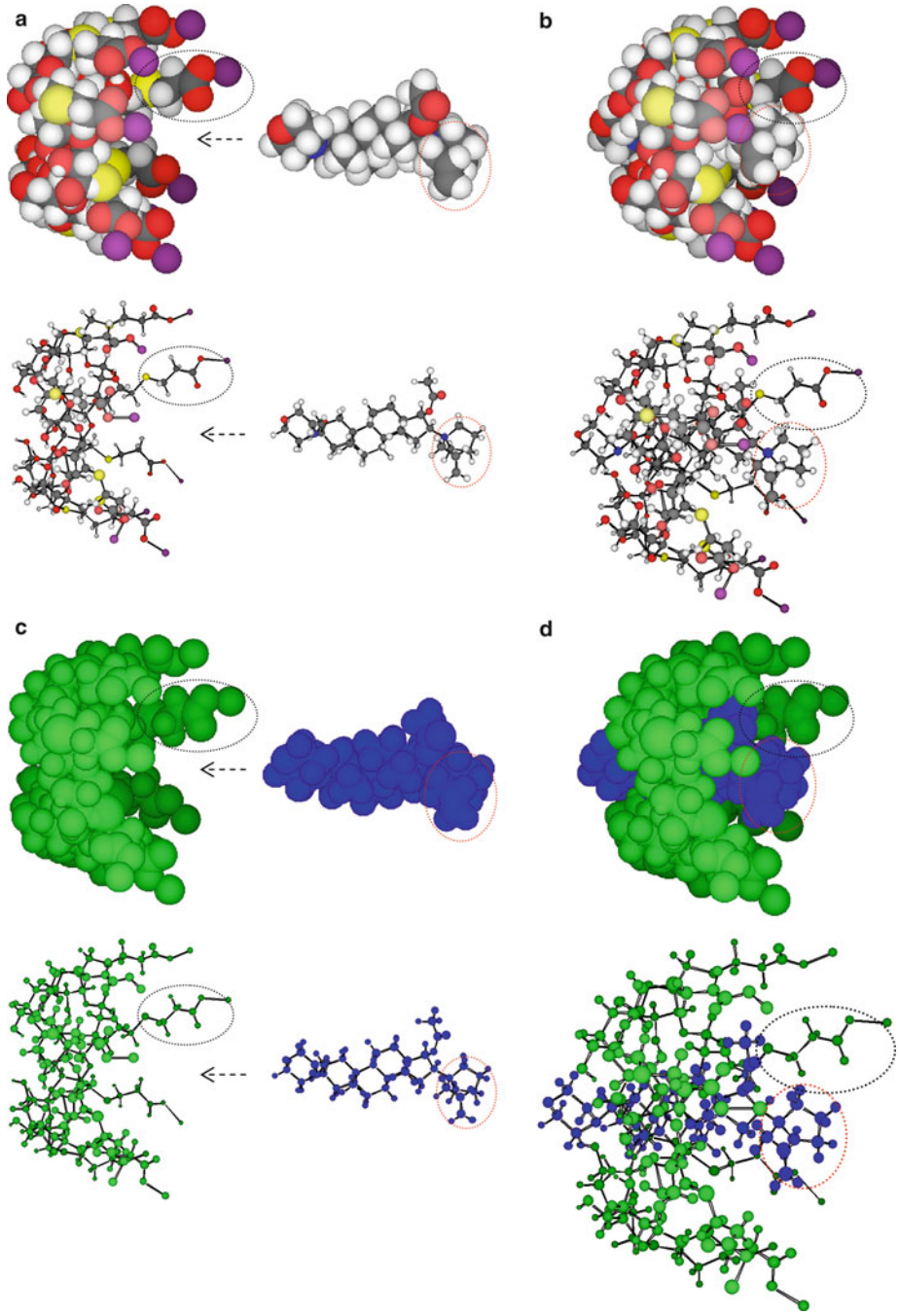


Fig. 7.17 Space-filling CPK and ball-and-stick three-dimensional molecular models (**a** and **b**, normal CPK coloring; **c** and **d**, mono-colors to distinguish molecules) illustrating the encapsulation of rocuronium by sugammadex to form a sugammadex–rocuronium inclusion complex. (**a**) Left-hand structure, sugammadex; right-hand structure, rocuronium. (**b**) Sugammadex–rocuronium inclusion complex. (**c**) Left-hand structure (*green*), sugammadex; right-hand structure (*blue*), rocuronium. (**d**) Sugammadex–rocuronium inclusion complex. The positively charged propenyl pyrrolidinium quaternary ammonium group on rocuronium (see Fig. 7.16) is *ringed in red* and one of the eight thio(2-carboxyethyl) sodium groups of sugammadex (see Fig. 7.15) is *ringed in black*. Substituents attached to ring A of the steroid backbone of rocuronium (see Fig. 7.16) are partially visible at the secondary rim (*left-hand side* here) of the cavity, rings B, C, and D of rocuronium are within the cavity, and the quaternary ammonium group attached to ring D is surrounded by carboxyl groups at the opposite, primary rim end (*right-hand side* here), of the sugammadex carrier. From Baldo BA et al. Drug-specific cyclodextrins with emphasis on sugammadex, the neuromuscular blocker rocuronium and perioperative anaphylaxis: implications for drug allergy. Clin Exp Allergy 2011;41:1663. Reprinted with permission from John Wiley and Sons

decrease pain when injected, but the effectiveness of the binding led to the application of sugammadex for the reversal of rocuronium-induced blockade by sequestering the drug and removing it from the neuromuscular junction.

7.4.6.2 Rocuronium, Sugammadex, and Anaphylaxis

Coincident with rocuronium's popularity to induce neuromuscular blockade has been an increase in reports of anaphylaxis to the drug and the suggestion that sugammadex, as well as rapidly reversing neuromuscular block, might also offer a novel treatment to reverse anaphylaxis caused by rocuronium. However, because the allergenic ammonium group of the encapsulated rocuronium molecule is visible at the primary face of the sugammadex carrier, doubts were expressed that sequestered rocuronium molecules would be protected from interacting with IgE antibodies. Case reports quickly appeared apparently supporting the suggestion that sugammadex might be a new and useful treatment to manage rocuronium-induced anaphylaxis and at the time of writing there are at least seven reports from six different countries of the mitigation of anaphylaxis provoked by the drug. Even so, the ammonium ion at position 16 on the steroid nucleus of encapsulated rocuronium is loosely surrounded by (2-carboxyethyl)thio groups of sugammadex, and with the tertiary ammonium group partly visible at the opposite end of the inclusion complex, both potentially reactive ammonium groups might still be accessible for binding with complementary IgE molecules (Figs. 7.16 and 7.17). These are questions that should be amenable to experimental in vitro investigation in the laboratory. In the meantime, since challenge studies with encapsulated rocuronium or induction of hypersensitivity with the NMBD followed by administration of sugammadex are not ethically permissible, further information on the effectiveness or otherwise of sugammadex in mitigating rocuronium-induced anaphylaxis will have to await the accumulation of more case reports.

7.4.6.3 Relative Affinities of the Interaction of Rocuronium with Sugammadex and IgE Antibodies

With an association constant K_a of 1.8×10^7 M^{-1} sugammadex forms a stable complex with its guest molecule rocuronium. There is no information available on the affinities and dissociation constants of IgE antibody–rocuronium complexes, but the average association constants for multideterminant, multivalent allergens such as pollen proteins for example with their complementary IgE antibodies are often high and in the range 10^{10}–10^{11} M^{-1}. By virtue of their tertiary and quaternary ammonium groups, NMBDs are probably at most bideterminant and bivalent and one might therefore expect lower average affinities (in terms of the average association constants) and avidities (the overall stability or strength of the complex) for rocuronium–serum IgE antibody interactions. In fact, the affinities and avidities of rocuronium–IgE complexes may be even lower than first expected because of the specificity of the drug–antibody interaction. The absolute specificities of NMBD-reactive IgE antibodies in patients' sera are not known since the source of the sensitizing agent(s) is also unknown, but, whatever the original antigenic stimulus, it is probably not an NMBD. This in turn leads to antigen–antibody combining site complexes of poorer complementarity or "fit" than seen in reactions between antibody combining sites and the antigen that stimulated the production of the antibody in the first place. If sugammadex is to successfully mitigate an ongoing anaphylactic response induced by rocuronium in a patient, its association constant for reaction with the NMBD has to be higher than the average association constant of the patient's IgE antibodies for rocuronium. Affinities for antibodies reacting with the same hapten can differ by up to a factor of 10^5 so the effectiveness of sugammadex in reversing rocuronium-induced anaphylaxis in different patients may vary. In general though, one may predict that higher affinities for the IgE–rocuronium complexes than for the

sugammadex complex would result in the failure of sugammadex administration to mitigate a reaction while mitigation of anaphylaxis would follow if the sugammadex–rocuronium affinity were higher.

7.4.6.4 Allergy to Sugammadex

Sugammadex itself may occasionally cause anaphylaxis and the withholding of approval by the United States Food and Drug Administration in 2008, even though the drug is approved for use in Europe, was due to concerns about allergic reactions to the modified cyclodextrin. In a recent report of such a reaction, intense erythema of the thorax, severe lip and palpebral edema, a fall in blood pressure, tachycardia, and bilateral wheeze were reported in a young adult 1 min after receiving a low dose of sugammadex (3.2 mg/kg). The patient proved skin test positive to sugammadex. Three other cases of sugammadex-induced hypersensitivity were recently reported from Japan when the drug was administered at doses ranging from 1.9 to 2.2 mg/kg. In one case, facial erythema and blepharedema developed 3 min after sugammadex and the patient was skin test positive to sugammadex. The second patient developed hypotension and generalized erythema 3 min after the cyclodextrin and again the skin test proved positive. The third patient, not skin tested, responded with wheeze and intense erythema 4 min after sugammadex. The three reactions occurred out of a total of 1,864 instances in which sugammadex was administered during general anesthesia. Sugammadex-specific IgE antibodies were not directly identified in these cases which might have been due to direct drug-induced mediator release. It has been pointed out that the use of host molecules such as sugammadex, other cyclodextrins, dendrimers, vesicles, cell ghosts, hydrogels, and so on to "carry" other molecules and improve drug delivery will sometimes produce changed immunological recognition including the potentiation and reduction of allergenic properties. There will be a need to take this into account in preclinical drug safety assessments and the possibility of altered allergenic behavior of even some well-known drugs should not be overlooked by allergists and dermatologists.

7.4.7 Antigenic Similarity Between α-Bungarotoxin and Neuromuscular Blocking Drugs

Elapid snake neurotoxins bind specifically and with high affinity to acetylcholine receptors on muscle cells and the electroplax of electric fishes. α-Bungarotoxin (α-BT) is a 74 amino acid (MW 7,983 Da) basic poypeptide α-neurotoxin from the Taiwanese banded krait that binds irreversibly and competitively to the nicotinic acetylcholine receptor at the motor end plate producing a non-depolarizing block of neuromuscular transmission. Competitive NMBDs like *d*-tubocurarine also act at the postjunctional membrane blocking the transmitter action of acetylcholine. Investigations of the affinities of NMBDs for the α-BT binding sites at the α-subunit of the acetylcholine receptor showed that the drugs had some of the highest affinities for the binding sites, in fact, even higher than acetylcholine and nicotine. These findings suggested that the NMBDs and α-BT probably share some properties of, for example, shape, conformation, and charge. The possibility that a similarity between the widely chemically different ligands might be detected immunologically was therefore investigated in competitive binding studies with sera from 16 different patients who experienced anaphylaxis to NMBDs. For two patients, both of whom reacted to succinylcholine, α-BT clearly inhibited the binding of IgE antibodies to a choline-solid phase, but little or no inhibition was seen when triethylcholine-, alcuronium-, *d*-tubocurarine-, or vecuronim-solid phases were used. For one patient, *d*-tubocurarine was the most potent inhibitor (IC_{50} 0.72 nmol), alcuronium was the weakest (IC_{50} 58 nmol) and α-BT was intermediate in inhibitory potency (IC_{50} 16 nmol) but more active than choline and triethylcholine (IC_{50} 23 and 20 nmol, respectively). Fifty percent inhibitory concentrations for succinylcholine, decamethonium, gallamine, and pancuronium were 9.8, 1.9, 8.4, and 7.7 nmol, respectively. For the second serum, a similar inhibitory pattern was seen with α-BT being more potent than alcuronium while

decamethonium and *d*-tubocurarine were the best inhibitors. Competition between polypeptides and small ligands at protein binding sites are known with nucleases and proteases. Changeux postulated that the sizes of α-BT (diameter 27 Å) and NMBDs such as *d*-tubocurarine (distance between nitrogens 14 Å) did not rule out the possibility of competitive binding at the cholinergic receptor. Further investigation of the possible antigenic similarity between the peptide toxin and NMBDs seems warranted. Identification of sera showing clear IgE antibody recognition of α-BT might prove useful in attempts to better define and ultimately understand the different and often puzzling antibody NMBD recognition patterns from different patients allergic to the same NMBD.

7.5 Anaphylaxis to Hypnotic Drugs Used in Anesthesia

Table 7.1 shows that amongst the anesthetic agents used in the years covered by the main surveys of anaphylactic responses, the Cremophor-based drugs alfathesin and propanidid contributed significantly, at least in the Australian series, to the list of induction agents causing reactions with an incidence as high as 1 in 875 cases. The drugs that replaced them, etomidate and propofol, rarely produce severe hypersensitivity reactions and that is also true for ketamine and midazolam. Therefore, because the Cremophor-based induction agents are of little or no relevance today and allergic or allergic-like reactions to etomidate, ketamine, and midazolam are so few, only the commonly and widely used propofol will be considered here along with the long-standing induction agent thiopentone.

7.5.1 Thiopentone

Used in the induction phase of anesthesia, thiopentone is an ultrashort acting barbiturate. The drug acts within 30–45 s producing its peak concentration in the brain about 1 min after injection. Thiopentone has now been largely replaced in anesthesia by propofol, but it is still the classic drug used in rapid sequence inductions and it is sometimes used in electroconvulsive therapy.

7.5.1.1 Incidence of Reactions, Clinical Features, and Epidemiology of Anaphylactic Reactions to Thiopentone

Hypersensitivity reactions to thiopentone are generally regarded as rare, with incidences of anaphylaxis of about 1 in 22,000 and 1 in 29,000 suggested in two prospective studies and only 1 in 400 in a third. After its introduction in 1934, 41 cases of anaphylaxis were attributed to the drug in 1980, only 13 published cases were identified in 1974 while 2 years later it was concluded that 30 cases had occurred since the drug's introduction. In 1985 in France 258 cases of anaphylaxis were identified in a literature search. The true incidence of allergic reactions to thiopentone is therefore not clear, but it seems likely that after the many millions of injections of the drug given annually, many cases of anaphylaxis may have gone unrecognized or been misdiagnosed.

Unlike NMBDs, previous exposure to thiopentone is a predisposing factor in producing anaphylaxis. Anaphylaxis on first exposure is unusual; at least six exposures are usually required and up to 37 uneventful exposures have been reported prior to the first reaction. Patients who react to thiopentone are older than reactors to NMBDs (43 vs. 36 years), women predominate with a female to male ratio of three to one, and the incidences of a history of allergy (42 %), atopy (36 %), and asthma (22 %) are similar to NMBD reactors. Also, unlike NMBDs, hypersensitivity reactions ranging from rashes to urticaria and severe exfoliative dermatitis have been seen with thiopentone.

7.5.1.2 Diagnosis of Immediate Allergic Reactions

7.5.1.2.1 Challenge Tests

Challenge tests with thiopentone have been used to confirm the diagnosis of anaphylaxis to the drug. Results obtained in challenge tests on two patients give some indication of the responses that might be expected. After building up the

challenge dose of thiopentone to 64 mg in a female patient 4 weeks after an anaphylactic-like reaction to the drug, the patient experienced "head spinning," turned bright red with erythema spreading down the body, the conjunctivae became injected and the pulse rate rose abruptly to 135. Administration of incremental increases of thiopentone up to a dose of 220 mg to an adult male who experienced a suspected anaphylactic reaction to thiopentone and was skin test negative to the drug produced severe itching and erythema within 17 min. A systemic reaction following a skin test with thiopentone has also been reported. Therefore, although challenge tests can and have been used to confirm diagnosis, their use should probably be reserved for patients in remote areas where other tests are unavailable or for cases where other tests are negative. This conclusion is reinforced by the fact that other useful and reliable tests are available to diagnose sensitivity to thiopentone.

7.5.1.2.2 Skin Tests

Diagnosis of suspected anaphylaxis to thiopentone generally rests on skin testing, a procedure that has proved its applicability. In a prospective skin test study on 83 patients, no significant differences were seen between prick testing and the intradermal test. Prick testing is performed with undiluted ready-prepared drug solution, that is, 25 mg/ml, while for intradermal testing, testing starts at a 1 in 10,000 dilution and proceeds up to a maximum of 1 in 10, that is, a concentration of 2.5 mg/ml maximum.

7.5.1.2.3 Serum IgE Antibody Tests

In the first clear-cut demonstration of the involvement of IgE antibodies in suspected anaphylactic reactions to thiopentone, the drug was covalently coupled to *bis*-oxirane-activated Sepharose and used in a solid phase radioimmunoassays with sera from patients who experienced anaphylaxis to the drug. Specificity of the binding of IgE antibodies in patients' sera to the drug-solid phase was demonstrated by inhibition of binding with free thiopentone; 5 μmol of drug generally produced up to 65 % inhibition of binding. Extensive quantitative inhibition studies with a number of selected barbiturate analogs identified two different allergenic determinants on opposite sides of the thiopentone molecule, the ethyl and secondary pentyl groups at position 5 of the pyrimidine ring nucleus and the region of the ring encompassing the attached sulfur atom (Fig. 7.18). Pentobarbitone, which differs from thiopentone only in the hetero atom (O for the former, S for the latter), was a key inhibitor in identifying the alkyl group determinants while good inhibition with 2-mercaptopyrimidine, and to a lesser extent thiouracil, served the same purpose in identifying the thio region as a second IgE antibody-binding structure. Although the immunoassay is a valuable supplement to skin testing for the detection of thiopentone-allergic sensitivity, the method can sometimes detect "false-positive" reactions and the interpretation of results is therefore not always completely straightforward. Sera from NMBD-allergic subjects containing high levels of IgE antibodies to substituted ammonium groups react with the thiopentone-solid phase, but this reaction is not inhibited by preincubation of the sera with NMBDs. Lability of thiopentone at the high pH used to prepare the drug-solid phase appears to be the explanation why the NMBD-reactive IgE antibodies bind in the assay. The molecular basis of this reaction was elucidated by further inhibition investigations. 2-Mercaptopyrimidine but not thiopentone or thiobarbituric acid inhibited binding of the NMBD-positive sera indicating that the ring nitrogens of the pyrimidine nucleus are the complementary binding structures for the NMBD-reactive IgE. These groups are presumably accessible to antibody binding on the drug-solid phase but sterically hindered on free thiopentone by the alkyl and, perhaps, keto groups. Table 7.12 summarizes the IgE antibody binding structures on the thiopentone molecule identified with sera from thiopentone-allergic patients and sera from patients allergic to NMBDs. Figure 7.19 shows diagrammatically how the pyrimidine ring nitrogens become accessible for antibody binding once the bulky alkyl and keto groups are removed.

7.5.1.2.4 Leukocyte Histamine Release

Injection of anesthetic doses of thiopentone into healthy, nonallergic humans leads to release of histamine into the plasma, but the amount is

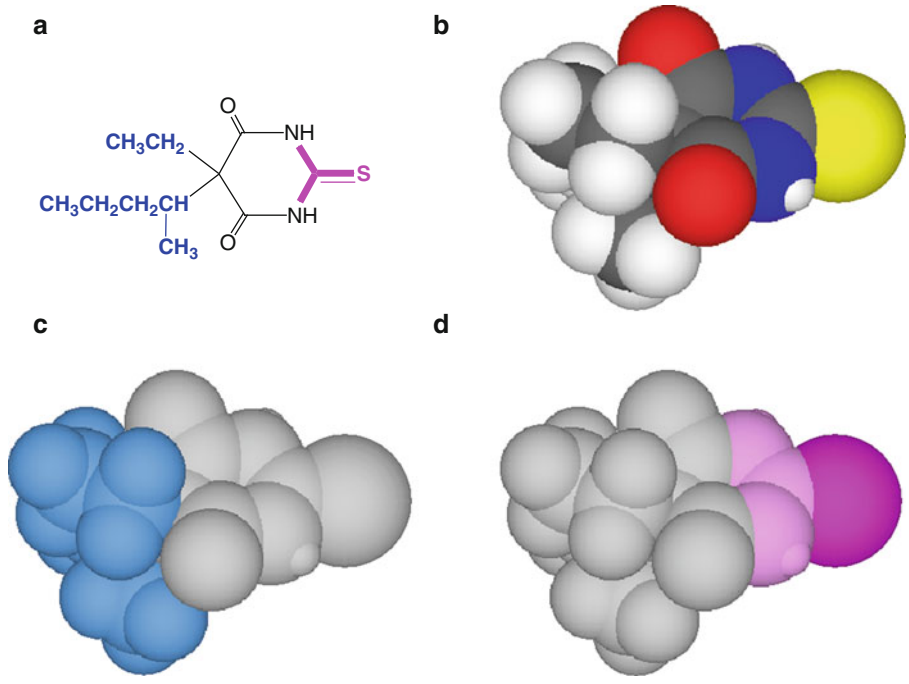
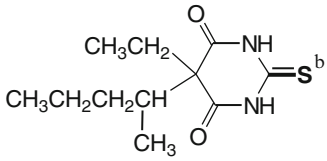
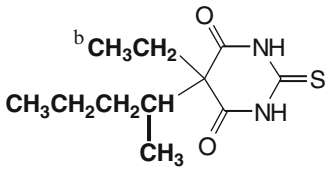
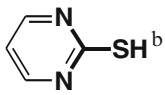


Fig. 7.18 Two-dimensional chemical structure (**a**) and CPK space-filling model (**b**) of thiopentone showing two different allergenic determinants identified on opposite sides of the thiopentone molecule, the ethyl and secondary pentyl groups at position 5 of the pyrimidine ring (shown in *blue*; **a** and **c**) and the region of the ring encompassing the attached sulfur atom (shown in *magenta*; **a** and **d**)

Table 7.12 IgE antibody-binding structures identified so far on thiopentone

Dominant structure in IgE-binding determinant[a] (in bold font)	Serological findings[c]	Clinical relevance
	Free drug (thiopentone) inhibits binding of patient's serum to "thiopentone"-solid phase[d]	Presence of IgE of this specificity indicates allergy to thiopentone
	As above	As above
	2-Mercaptopyrimidine[e] unlike thiopentone inhibits binding of IgE to "thiopentone"-solid phase[d]	Reactive IgE in sera from some NMBD-allergic subjects. Subjects not allergic to thiopentone

From Baldo BA & Pham NH. Structure–activity studies on drug-induced anaphylactic reactions. Chem Res Toxicol 1994;7: 703. Reproduced with permission from American Chemical Society

[a]Exact confines of determinant not always clearly defined with all sera

[b]Dominant feature of determinant shown. Other structures, in particular the pyrimidine ring, probably has an auxiliary function

[c]Findings when used with sera from subjects allergic to thiopentone or an NMBD

[d]Thiopentone is used to prepare drug-solid phase, but coupling conditions probably lead to some decomposition of the thiopentone. Exact structure of attached species is therefore uncertain

[e]2-Mercaptopyrimidine also inhibits binding of IgE antibodies reactive with the thio region of thiopentone

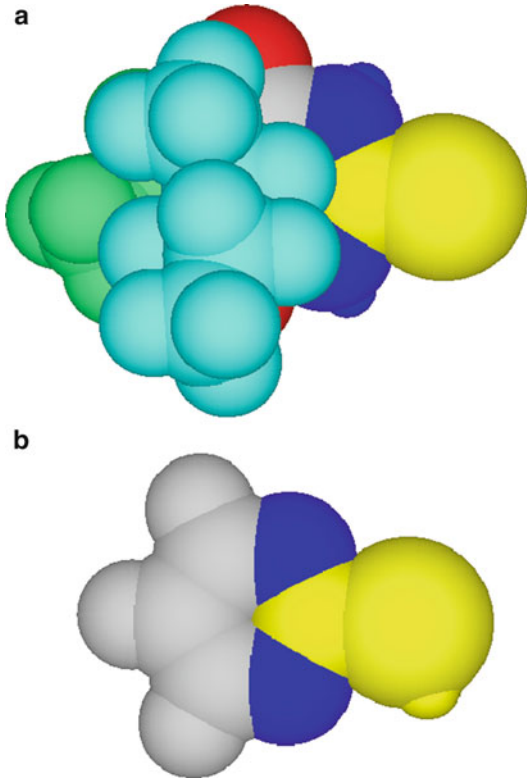


Fig. 7.19 Space-filling models showing how the pyrimidine ring nitrogens become exposed and accessible for antibody binding once the bulky alkyl and keto groups are removed from thiopentone. Models for (**a**) thiopentone and (**b**) 2-mercaptopyrimidine. Removal from thiopentone of the bulky secondary pentyl group (*light blue area*) and the ethyl (*green*) and two keto groups (*red*), the latter two partially obscured in (**a**), allows accessibility to the ring nitrogens (*dark blue*). The nitrogens are clearly visible in (**a**), but the view from the opposite side of the molecule shows that accessibility to the nitrogens is impeded by the keto groups. In (**a**), the nitrogens show the attached hydrogens which are absent in (**b**). The large sulfur atom (*yellow*) is freely accessible from both sides of the molecule

relatively small and too little to affect the circulatory system. Thiopentone-induced histamine release from leukocytes has been studied in vitro by a number of groups using leukocytes from subjects who reacted to the drug and to barbiturate analogs for cross-reactivity studies. In one study, pentobarbitone also produced histamine release, but methohexitone did not. In keeping with the in vivo findings, thiopentone induced only a moderate increase in histamine from peripheral blood leukocytes taken from a patient who experienced a life-threatening reaction to the drug. Thiopentone at a concentration as low as 7×10^{-6} M released histamine from leukocytes taken from a patient who reacted to the drug. Serum from the patient failed to passively sensitize cells from five different control subjects. This was interpreted as evidence for an anaphylactoid rather than an IgE-mediated anaphylactic reaction.

7.5.2 Propofol

Propofol, 2,6-diisopropylphenol, is administered intravenously and used in general anesthesia as a short-acting induction and maintenance agent. The drug is formulated as an oil–water emulsion containing 1 % propofol, soybean oil 10 %, glycerol, and egg phospholipid as emulgent.

7.5.2.1 Propofol and Anaphylaxis

Although propofol is generally said to be a remarkably safe drug, 14 patients were reported to have life-threatening reactions within a few minutes of the administration of propofol, numerous other anaphylactic-like reactions have been reported, and other occasionally observed hypersensitivity reactions include bronchospasm, angioedema, urticaria, and erythematous rash. The overall incidence of anaphylaxis induced by propofol in France is about 1 % and 0.65 % in the Australian survey (Table 7.1). Another survey estimated that 1.2 % of cases of perioperative anaphylactic shock were attributable to propofol. Risk factors for a reaction are said to be a history of previous drug allergy and the use with atracurium, the latter because of possibly enhanced histamine release.

The picture of propofol's involvement in evoking anaphylaxis is confusing with some aspects of the suggested underlying mechanism open to doubt. In the study of the 14 patients with life-threatening reactions, positive intradermal skin tests were obtained in eight patients at concentrations of 10 μg/ml propofol or less, and because a concentration of 100 μg/ml had been shown to be negative in 100 patients who showed no adverse reaction to the drug, the eight positive

tests were regarded as an indication of the presence of specific IgE antibodies. In an attempt to detect IgE antibodies to propfol, Phenyl-Sepharose® was used as a solid support to bind the drug to the support by hydrophobic interaction. After incubation of the propofol-Sepharose-solid phase with patients' sera, uptakes of IgE antibody were indeed observed, but background binding levels and binding of NMBD-reactive IgE in sera from some subjects were also alarmingly high, suggesting that some nonspecific binding might be involved. Significantly, no ionic detergent was included in the washing procedures during the assay and no steps (other than the addition of 1 M sodium chloride) specifically directed at minimizing nonspecific binding appear to have been undertaken. A direct and convincing way to establish specificity of a drug–antibody reaction is by inhibition experiments, but this approach was precluded by propofol's insolubility in aqueous media. In interpreting the results of the study, it was concluded that one positive test was sufficient to indicate an IgE-dependent mechanism and, on this basis, 13 of the 14 patients had therefore experienced a true anaphylactic type I allergic reaction. Some years after this study, the same investigators announced that the solid phase drug-Phenyl-Sepharose IgE immunoassay for propofol "has to be reconsidered as non-specific binding of hydrophobic drugs." It was also said that propofol and NMBDs may potentiate mediator release by a "non-elucidated mechanism," but what that mechanism might be remains obscure.

7.5.2.2 Skin Tests

Prick testing with propofol is said to be unreliable and intradermal testing is recommended. Prick testing is performed with the undiluted propofol formulation (10 mg/ml). For intradermal testing, the maximum concentration is a 1 in 10 dilution, that is, propofol 1 mg/ml. Testing should start at 1 in 1,000 and proceed up to the 1 in 10 dilution.

7.5.2.3 Chemical Structure and Allergenicity

The hydrophobicity of propofol is due to the aromatic phenyl nucleus and the two isopropyl groups on opposite sides of the six-membered ring structure (Fig. 7.20). It has been speculated that the isopropyl groups confer allergenic divalency on the molecule, and although this has been repeated many times in the literature, there is as yet no convincing evidence that it is so.

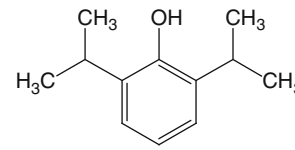


Fig. 7.20 Chemical structure of propofol. The aromatic ring nucleus and two isopropyl groups give the molecule its hydrophobic properties

7.5.2.4 Propofol and Allergies to Egg and Soybean Oil

In 1994 an allergic reaction to propofol was reported in an egg-allergic patient. This led to the suggestion that egg allergy might be a possible risk to consider prior to propofol administration. Following the hospitalization for treatment of respiratory symptoms of a 14-month-old boy with a history of airways disease and allergies to egg, peanut, and molds, propofol was administered for sedation. The condition progressed to anaphylaxis with the patient becoming hypotensive and tachycardic. Because the propofol formulation contains both soybean oil and egg phospholipid, it was concluded that, if possible, propofol should be avoided in patients with allergies to egg and/or soybean oil. In a recent retrospective review of cases for the period 1999–2010 of children with egg and/or soy allergy, 28 egg-allergic subjects with a total of 43 propofol administrations were identified. No soy-allergic children were found. One non-anaphylactic immediate reaction 15 min after propofol administration was identified in a 7-year-old boy with a history of egg anaphylaxis and multiple other IgE-mediated food allergies. A skin prick test to propofol (performed within 12 months of the reaction) was positive with a wheal of 3 mm. It was concluded, "Propofol is likely to be safe in the majority of egg-allergic children who do not have a history of egg anaphylaxis." The discovery

of the single patient with egg allergy who reacted to propofol more or less guarantees that egg-allergic sensitivity will continue to be regarded as a risk factor that cannot be ignored when propofol is administered. Rather than singling out egg, some have suggested that patients with multiple food allergies including soy, and a history of eczema and asthma, might increase the risk of allergy to propofol.

In Asia allergies to soy are well known and an association of soybean allergy with propofol-induced anaphylaxis has been suggested. In a recent convincing case, anaphylaxis with severe oropharyngeal edema and bronchospasm occurred in a 74-year-old woman with a history of soy allergy a few minutes after receiving propofol. Skin prick tests revealed positive reactions to propofol and 20 % Intralipid® which, like the propofol formulation, contains soybean oil. It was concluded that the anaphylactic reaction was caused by the soybean oil present in the administered propofol.

7.6 Anaphylaxis to Colloids

The risks associated with blood transfusions and the shortage of available blood have led to increased administration of substitute solutions for volume expansion. Colloids are commonly used and three of the main colloids commonly administered, gelatin, hydroxyethyl starch, and dextran will be discussed. Each one may cause adverse reactions ranging from grade I with skin manifestations to grade IV involving cardiac and/or respiratory arrest.

7.6.1 Hydroxyethyl Starch

Hydroxyethyl starch (HES), also known as hetastarch, is most often administered for intravascular volume expansion during the perioperative period and for resuscitation from trauma and shock. Because of its property of increasing blood fluidity, hydroxyethyl starch is sometimes infused to treat patients with disturbed microcirculation.

7.6.1.1 Chemistry

HES, a nonionic preparation, is synthesized from amylopectin and is made up of a backbone of d-glucose units linked α-(1-4) and with branches of d-glucose units attached by α-(1-6) glycosidic bonds. Hydroxyethyl groups are attached at glucose carbons 2, 3, and 6 (when free). The attached hydroxyl groups retard hydrolysis of the polysaccharide by amylase, thereby delaying its elimination from the circulation. The degree of branching of a HES polymer refers to the ratio of α-(1-6) branches to d-glucose units, and the degree of substitution, generally expressed as a number between 0 and 1, gives the fraction of d-glucose units bearing a hydroxyl group. For example, a degree of branching of ratio 1:20 signifies one α-(1-6) branch for every 20 d-glucose units; a degree of substitution of 0.7 indicates seven hydroxyethyl groups for every 10 d-glucose units. HES can be monodisperse, that is, molecules of one molecular weight only, or polydisperse where a range of molecular weights make up the colloid. For polydisperse colloids, molecular weight can be expressed as the weight average molecular weight Mw or the number average molecular weight Mn. These give different number values, for example, pentastarch range 10–1,000 kDa, Mw 280, is equivalent to Mn 120.

7.6.1.2 Risk and Adverse Reactions

HES is well tolerated with an incidence of adverse events less than gelatin and dextran. One report estimated the incidence of allergic reactions to be 0.0004 % while figures for the risk of life-threatening reactions are in the region of 0.006–0.085 % (1 in 1,172). For comparison, the risk of anaphylactoid reactions to albumin is 0.011 %. Clinical manifestations of reactions include anaphylactic and anaphylactoid shock, erythema, urticaria, and pruritus, the latter having an incidence of 10–40 %. In a prospective, randomized, controlled study, HES 6 % (200/0.5) and Ringer lactate solution were compared for the induction of anaphylactoid reactions and pruritus. Results showed no differences although there was an incidence of

more than 10 % for pruritus in both groups. Pruritus, which may persist for up to 1–2 years, is generally refractory to usual treatments and is increasingly being recognized as a common, frequently severe, and protracted adverse effect of HES administration. It has been claimed that all currently available HES solutions of diverse molecular weights and substitutions are subject to the risk of pruritus, thought to result from the deposition of HES in tissues particularly macrophages. Because of the relative infrequency of reactions to HES and the usual difficulties of identifying an allergic reaction and the causative agent during the perioperative period, it has been pointed out that HES may be overlooked as a cause. There are some reports in the literature that bear that out.

7.6.1.3 Reaction Mechanisms and Diagnosis

The possibilities of antibody formation by patients given HES and involvement of antibodies in adverse reactions to the agent have been investigated in a small number of studies. In one investigation of just over 1,000 patients 14 days after HES administration, antibodies of the IgM class were found in only one patient, and despite repeated HES infusions, no clinical reaction eventuated. The investigators concluded that antibodies to HES are extremely rare and they do not necessarily provoke an anaphylactic response. In a similar study of 1,056 patients, the investigators concluded that preformed antibodies to HES do not exist in humans or are extremely rare. A claim to have detected anti-HES IgE antibodies was made following retrospective testing of sera from an anaphylactic episode. Details of the patient's exposure and specificity of the antibody are lacking. Pentastarch-specific antibodies, presumably IgE, that bound to and activated basophils in the BAT were found in the serum of a woman investigated for a severe anaphylactic/anaphylactoid episode.

For skin testing, solutions are used undiluted for the prick test and at a dilution of from 1 in 100 to undiluted for intradermal testing.

7.6.2 Gelatin

Gelatin is a protein obtained by hydrolysis of animal (usually cow and pig) collagen from skin, bone, and connective tissue. Allergic reactions to gelatin have been reported after eating flavored fruit gums and condiments, following injection of vaccines containing gelatin as a heat stabilizer and after infusion of plasma expanders containing the protein. The overall frequency of anaphylactoid reactions (grades I–IV) to colloid intravascular infusions containing gelatin was estimated to be 0.115 % and for severe reactions (grades III and IV) 0.038 %. A more recent estimate for severe reactions gave a figure of 0.345 % for gelatin. Risk factors for allergy to gelatin include allergy following ingestion of the protein and drug allergy in general. Male gender has also been mentioned.

As a blood volume expander, gelatin has been or is marketed as Haemaccel® and Gelofusine®. Both are derived from bovine spongiform encephalitis-free bovine herds in the USA, Haemaccel is cross-linked with urea and has a molecular weight of about 35,000 Da, and Gelofusine is succinate-linked with a mean molecular weight of about 30,000 Da. Each has been associated with reports of allergic reactions and at least one study has demonstrated allergic cross-reactivity between the two preparations. Clinical manifestations of reactions to gelatin include anaphylaxis, sneezing, bronchospasm, and urticaria. The mechanism of allergic reactions to gelatin colloids has been said to be IgE antibody and non-IgE antibody mediated and diagnosis has been based on skin testing which, for intradermal testing at least, carries its own risk of anaphylaxis. Recently, the BAT has been successfully applied to confirm anaphylaxis and, together with positive skin tests, the involvement of gelatin-reactive IgE antibodies. A Phadia ImmunoCAP® test for the detection of IgE antibodies to gelatin is also available. Direct histamine release by gelatin has been proposed to explain some reactions to the protein.

For skin test diagnosis, gelatin solutions are used undiluted (for Gelofusine, Haemaccel, and gelatin solutions ~35 mg/ml) in the skin prick test and at dilutions of from 1 in 1,000 to 1 in 10 for intradermal tests.

7.6.3 Dextrans

Dextrans are polysaccharides of varying chain lengths synthesized from sucrose by lactic acid bacteria *Leuconostoc mesenteroides* and *Streptococcus mutans*. Dextran chains are composed of d-glucose units linked α-(1-6) with branches linked α-(1-3). Two intravenous solutions containing the high molecular weight dextrans 40 and 70 are used for plasma volume expansion in the treatment of hypovolemic shock, for postoperative thromboembolic prophylaxis, as a component of the pump prime for cardiopulmonary bypass, and to promote blood flow in the microcirculation. In a large-scale multicenter study involving nearly 201,000 infusions of intravascular colloid volume substitutes, the overall frequency of anaphylactoid reactions (grades I–IV) for dextrans was 0.032 %, while for severe reactions (grades III and IV) the figure was 0.008 %. In a more recent prospective screen of nearly 20,000 patients, the incidence of anaphylactoid reactions to dextrans was 0.273 %. Reports to the FDA from 1969 to 2004 of adverse events involving dextrans numbered 366, one quarter of which was anaphylactic/anaphylactoid in nature. Anti-dextran IgG antibodies cross the placenta so dextran administration should normally be avoided in pregnant women. Cases of neurological impairment and deaths in neonates following anti-dextran administration have been reported. Identified risk factors are few with atopy linked to milder reactions and high levels of anti-dextran antibodies associated with severe reactions. Clinical signs and symptoms range from anaphylactic shock, fever, and death to nausea and minor flushing. Cutaneous symptoms include urticaria, pruritus, angioedema, and macular rash. Respiratory manifestations are wheezing, chest tightness, coughing, dyspnea, and pulmonary edema.

A well-known side effect since the mid–late 1940s is dextran-induced anaphylactic reactions (DIAR) that can range in severity from mild erythema (grade I) to death (grade V). DIARs are caused by the formation of immune complexes formed by reaction of preexisting circulating antibodies to dextran, primarily of the IgG class, with the injected dextran. In grade III and IV reactions at least, the immune complexes formed from the cross-linking of dextran by the dextran-specific IgG antibodies bind to receptors on the cell surfaces of mast cells and basophils leading to the release of mediators that cause circulatory collapse and bronchoconstriction. In the field of quantitative immunochemistry dating back to the foundations of the modern science of immunology, dextran has its own special place as a model antigen in the study of antibody–carbohydrate reactions and for its central role in experiments that defined the size of the antibody combining site. By the early 1950s, a project group led by Elvin Kabat in New York and carried out with the backing of the Office of the Surgeon General, US Army, had shown that significant correlations existed between: (1) skin test sensitivity and allergic reactions to some dextrans; (2) the presence of antibodies to some dextrans and systemic allergic reactions; and (3) the structural complexity of some dextrans and the incidence of positive skin tests in the general population. It was further demonstrated that partial hydrolysis of native dextrans produced a reduction in their capacity to produce a positive wheal and flare reaction and that a low proportion of non-1-6 linkages and elimination of higher molecular weight fractions produced a low incidence of systemic allergic reactions. By the 1970s, accumulated knowledge of the humoral recognition of dextran polysaccharides and oligosaccharides of varying sizes laid the groundwork for an animal model of DIAR that showed that the hapten dextran 1 (molecular weight 1,000 Da), administered immediately before dextran 40, greatly reduced the incidence of severe hypotension. The mechanism of this effect is due to the monovalent hapten nature of dextran 1 that interacts with anti-dextran antibody combining sites

without forming an antigen–antibody lattice complex and therefore no detrimental immune complexes. This can be viewed as competitive hapten inhibition carried out in vivo. Subsequently, large multicenter clinical trials found that pre-injection of 20 ml dextran 1 reduced the incidence of severe DIAR from 25 cases per 100,000 units dextran 40/70 to three cases per 100,000 units. A review of the 10-year period 1983–1992 revealed that the prophylactic use of dextran 1as hapten was associated with a 35-fold reduction in the incidence of severe DIAR and a 90-fold reduction of lethal DIAR. It has been claimed that the introduction of dextran 1 for hapten inhibition prior to infusion of dextrans 40 and 70 has made these plasma volume expanders the safest of all the volume expanders in clinical use.

Adverse reactions to dextran 1 were investigated in trials involving over 70,000 patients. The following reactions and incidences were recorded: cutaneous reactions 0.016 %; moderate hypotension 0.014 %; severe hypotension 0.001 %; bradycardia and moderate hypotension 0.013 %; bradycardia and severe hypotension 0.001 %; bradycardia alone 0.004 %; and mild symptoms (nausea, pallor, shivering) 0.011 %.

For skin test diagnostic investigation, dextran (6–10 mg/ml) is used undiluted in prick tests and at a maximum concentration of a 1 in 100 dilution of the prick test concentration for intradermal testing.

7.7 Local Anesthetics

Local anesthetics are the key components that enable the application of local and regional anesthetic techniques for the treatment of acute pain and the drugs are also used in the management of chronic pain where they may have a prolonged effect. It can be said that local anesthetics revolutionized the practice of anesthesia, surgery, dentistry, and ophthalmology and with their daily routine use in countless minor procedures and their applications in obstetrics they are known to affect almost everyone at some time in their life. With regard to "allergy" to local anesthetics, it often seems that the general public and the medical profession perceive the situation differently. The public's perception is that local anesthetics are a frequent cause of reactions while allergists at least know that although these agents are well down any list of drugs causing true allergic reactions, immediate or delayed, referrals for local anesthetic allergy are often as high as for known allergenic drugs such as the penicillins.

7.7.1 Chemistry

All local anesthetics possess similar structural features of a hydrophobic aromatic ring linked to a hydrophilic amine group and the linkage group may be an ester, amide, ether, or ketone. In clinical practice today, the esters and amides predominate with the latter group by far the most widely used. Well-known esters are cocaine, benzocaine, procaine, and tetracaine and amides include lidocaine (lignocaine), prilocaine, etidocaine, mepivacaine, bupivacaine, ropivacaine, and articaine (Fig. 7.21). As with the sulfonamide drugs, all of the local anesthetic esters are derivatives of *p*-aminobenzoic acid (see Sect. 6.2.1.1), and this compound is released upon hydolysis. Prilocaine, etidocaine, mepivacaine, bupivacaine, and ropivacaine exist as stereoisomers (R-(+) and S-(−) isomers) and most are marketed as racemic mixtures, although ropivacaine is used as the S-isomer and S-bupivacaine is less toxic than R-bupivacaine.

7.7.2 Adverse Reactions to Local Anesthetics

Following the first description of "allergy" to a local anesthetic over 90 years ago, there was initially a steady stream of reports of reactions to the drugs consisting mainly of erythema or edema. With the introduction of the amide local anesthetics, the number of hypersensitivity reactions tapered off significantly, indicating that ester compounds were less well tolerated. Even today, however, reports of adverse reactions to local anesthetics occasionally appear, but the nature of the reactions cannot always be described

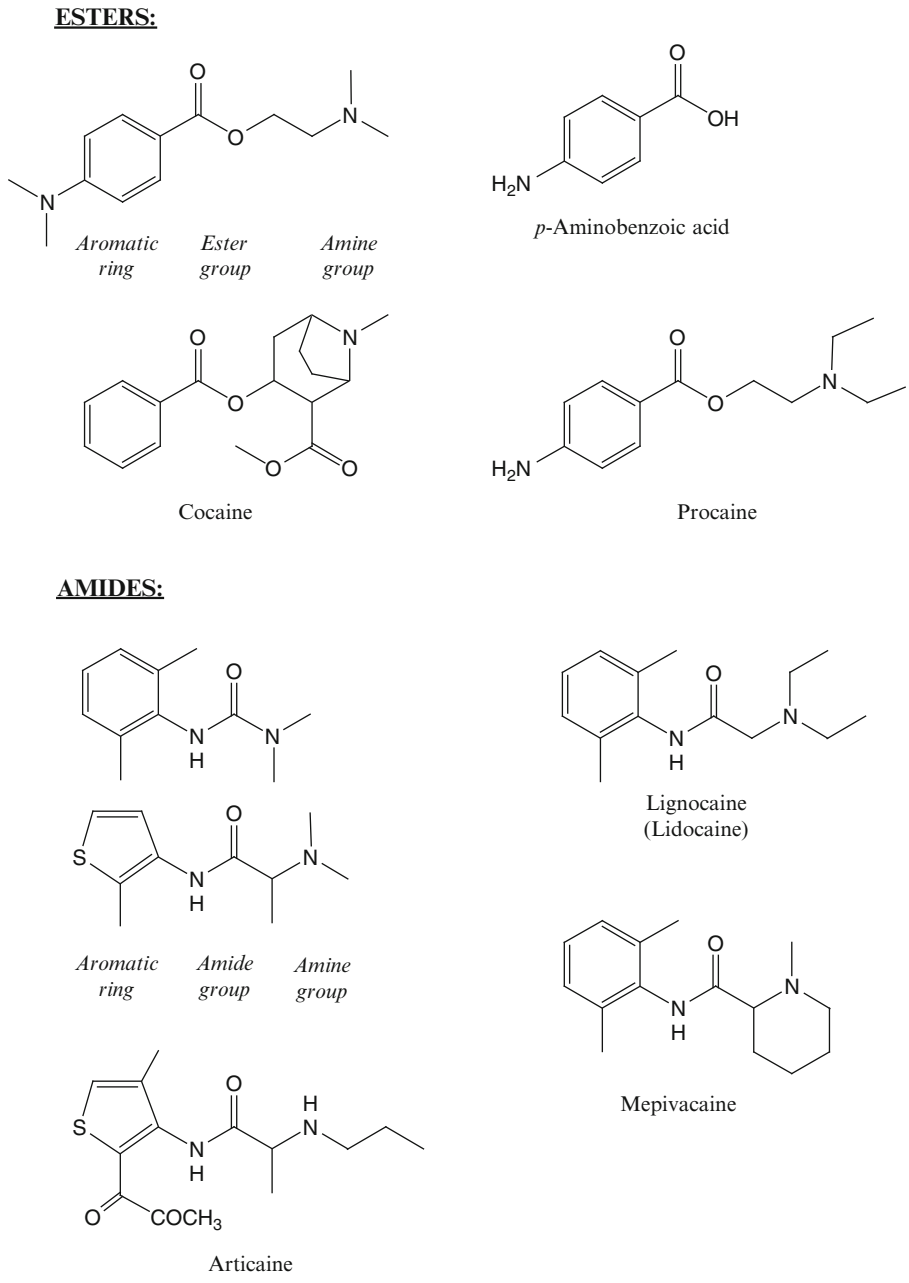


Fig. 7.21 Chemical structures of two main categories of local anesthetics, esters and amides, with some structures of important members of each category

as hypersensitivity responses. In fact, many of the reactions, if not the majority, may be vasovagal reactions, anxiety attacks, and some may be reactions to epinephrine which is frequently added to local anesthetics as a vasoconstricting agent for dental and some minor procedures. Apart from the presence of epinephrine, local anesthetics may evoke sympathetic effects that include palpitations, light-headedness, syncope, or tachycardia. Local anesthetics are heavily used in dental procedures and many reports of adverse reactions following their administration emanate

from that source. A prospective study of just over 5,000 patients who received local anesthetics during dental treatment revealed only 25 adverse reactions (~0.5 %) with none of the reactions allergic. Twenty-two of the 25 reactions were mild, vasovagal in nature, or what was described as quickly reversible psychogenic reactions. Other additives to formulations of local anesthetics such as parahydroxybenzoates (parabens) and the antioxidant sodium metabisulfite have come under suspicion as causative agents for adverse reactions. The former compounds (which are no longer added to many formulations) are known to cause contact sensitivities but, together with sodium metabisulfite, evidence of involvement in immediate reactions is lacking. The incidence of what has been called "systemic toxicity" to local anesthetics is said to have decreased from 0.3 to 0.01 % over the past 30 years.

7.7.2.1 Immediate Reactions

True type I immediate reactions to local anesthetics, especially the amides, are rare, but they have been documented even for the amides. A number of reactions, well short of 1 % of reported allergic reactions to local anesthetics, are thought to be immune mediated and, of these, amide compounds make up a small proportion. In perhaps the most comprehensive review of data related to local anesthetic allergy undertaken so far, reports in the French Pharmacovigilance and the Groupe d'Etudes des Reactions Anaphylactiques Peranesthesiques (GERAP) databases for the 12-year period 1995–2006 were analyzed for clinical features, skin test results, delayed- and immediate-type allergic reactions, and cross-reactions. Of 16 relevant cases identified, an immediate reaction occurred in 11 patients, lidocaine was the drug most involved (11/16), and cross-reactivity between the amides lidocaine and mepivacaine was found in six cases. Reactions occurred mostly in young females (F:M ratio 14:2) and diagnoses were confirmed with prick tests, intradermal tests, and challenges. The finding that lidocaine was the drug most often involved in immediate reactions is consistent with the fact that it is the most often used local anesthetic in medical practice. First symptoms occurred within 1 h of administration and included respiratory, cardiovascular, and neurological signs. It was concluded that both clinical history and skin testing are necessary to confirm an immediate reaction and, in fact, the latter test is considered mandatory. Five delayed-type reactions with mainly cutaneous signs of erythema, pruritus, urticaria, and eczema appearing 6 h to 1 month after local anesthetic administration were also identified in the French study. Type IV hypersensitivity reactions particularly to ester-type local anesthetics are well known and the involvement of amides in delayed reactions is also recognized. Of the six cross-reactivity cases mentioned above, four were type I reactions, and although cross-reactivity between ester local anesthetics is well known, this was not the case for the amides. Lack of cross-reactivity between articaine and the other amides is probably due to the presence of a thiophene and not a phenyl ring in the former drug (Fig. 7.21).

The rarity of immediate reactions to local anesthetics is further emphasized in results obtained in two large studies of a total of 354 patients with a history of reacting to the drugs. No evidence for an IgE-mediated reaction was found in one of the studies involving 157 patients while three patients, two with an immediate reaction and one with a delayed response, were seen in the review of the other 197 patients. No IgE antibodies were detected in the two immediate reactors.

7.7.2.2 Delayed Reactions

Lidocaine-specific T cell lines and clones generated from patients with contact dermatitis to the drug proved to be mainly MHS class II restricted and CD4+, but some were MHC class I restricted CD8+ clones. The T cell lines cross-reacted with mepivacaine but not with procaine, oxyprocaine, bupivacaine, and tetracaine. Most of the CD4+ clones produced a Th2-like pattern of cytokines with a high IL-5 component. Cellular recognition studies with the rather chemically nonreactive lidocaine showed that it was recognized directly by $\alpha\beta^+$ T cells in an HLA-DT restricted manner with neither drug metabolites nor breakdown products involved or protein processing required. Examination of the cross-reactivity between

lidocaine and mepivacaine at the cloned cell level showed cross-recognition with small structural changes affecting T cell stimulation. For example, one clone recognized lidocaine and mepivacaine but no hydroxy metabolite, others responded to 3-hydroxy metabolites, and only some amines and a broadly reactive clone tolerated hydroxy substituents and reacted to linear and cyclic amines. Findings with the broadly reactive clone suggested that the structure of the amine side chain of local anesthetics is essential for recognition by the T cell receptor.

7.7.3 Diagnosis of Reactions to Local Anesthetics

For skin testing, solutions of local anesthetics are used undiluted in the prick test and either at or up to a dilution of 1:10 in the intradermal test. A few positive intradermal tests have been reported with dilutions of 1:100 down to 1:10,000. Examples of some undiluted starting concentrations are lidocaine 10 mg/ml, bupicacaine 2.5 mg/ml, mepivacaine 10 mg/ml, and ropivacaine 2 mg/ml. Although it has been stated that skin testing in the diagnosis of hypersensitivity to local anesthetics is mandatory, it should be said that there is no universal agreement on the value and place of skin testing in the assessment of patients' adverse reactions to these drugs. A recent review of the literature over the period 1978–2009, focusing on sensitivity and specificity of skin testing and provocation challenge, found only three immediate reactors in 1,094 patients when the local anesthetic was represented. None of these three reactors were originally detected by skin prick or intradermal tests. Over all the studies, false-positive skin tests varied from 0 to 27 %. From such results, some have concluded that skin tests are a poor predictor of positive challenge since most adverse reactions to local anesthetics are not allergic in nature and skin tests are sometimes positive in patients who tolerate challenge with the suspected drug. For patients confirmed by positive challenge, the specificity and sensitivity of skin tests have also been questioned with claims (from small numbers) of only 43 % and 14 %, respectively, for the sensitivity of intradermal and prick tests. In addition to its "gold standard" status as a diagnostic test, provocation testing for the investigation of adverse reactions to local anesthetics has been described as safe and well tolerated.

IgE antibodies to local anesthetics appear to be so rare that some have even doubted that they occur, but occasional case reports indicate an underlying mechanism of type I hypersensitivity and some skin test results suggest that these antibodies do exist. Evidence from a dot-blot procedure for an IgE-mediated reaction to lignocaine has been presented, and more recently, IgE antibodies to mepivacaine were reliably detected by a Phadia immunoassay shown by inhibition studies to be specific.

7.8 Polypeptides

7.8.1 Protamine

Protamines are arginine-rich nuclear proteins with molecular weights in the range 4,500–5,000 Da. Generally purified from sperm heads in salmon milt and used as the sulfate, protamines are used to reverse heparin's anticoagulant effect via binding of its basic guanidine groups in arginine to acidic heparin molecules and to retard the absorption of insulin (as neutral protamine Hagedorn, NPH) and increase its duration of action. A study nearly 40 years ago on the cardiorespiratory effects of protamine after cardiopulmonary bypass surgery in humans showed that the protein at a concentration of 6 mg/kg produced a marked fall in cardiac output and a brief fall in systemic arterial pressure. As a result of its use during cardiac catheterization and cardiopulmonary bypass in very large numbers of patients, its interaction with heparin, and the occasional reactions it provokes, protamine's adverse effects have received a good deal of attention. From numerous clinical and experimental investigations it is known that protamine induces increased pulmonary artery pressures and falls in blood pressure, heart rate, cardiac output, myocardial oxygen consumption, and systemic vascular resistance.

7.8.1.1 Adverse Reactions to Protamine

When administered too quickly, protamine may cause transient flushing, a feeling of warmth, bradycardia, and hypotension and this has resulted in the practice of injecting the drug slowly, usually over a 5–10 min period. Adverse reactions to protamine can manifest as flushing, rash, urticaria, angioedema, wheezing, hypotension, bronchospasm, cardiovascular collapse, and sometimes death. Until the late 1970s, reactions to protamine were generally considered to be non-immunologic in nature and probably due to direct effects including mast cell degranulation. Experiments with human basophils and lung mast cells have not always clearly demonstrated histamine release by protamine or protamine–heparin complexes, but Marone's group in Naples showed that protamine released the preformed mediators histamine and tryptase from human basophils but did not stimulate de novo synthesis of eicosanoids. Protamine also caused the release of histamine and tryptase from human heart mast cells and, to a lesser extent, from synovial mast cells but not lung mast cells. As for basophils, protamine did not induce de novo synthesis of LTC_4 and PDG_2 from lung and skin mast cells. Fulminating non-cardiogenic pulmonary edema, also referred to as adult respiratory distress syndrome or ARDS, has been described as a rare event occurring in 0.2 % of cardiopulmonary bypass patients with mortality rates approaching 30 %. Protamine-induced direct release of histamine and/or complement activation is the suspected mechanism. What has been termed protamine-induced pulmonary artery vasoconstriction with cardiovascular collapse after cardiac surgery and thought to involve the potent vasoconstrictor thromboxane A_2 is reported to have an incidence of 1.5 %. This may be mediated by complement activation resulting from the interaction of protamine with heparin or anti-protamine IgG and C5-mediated thromboxane generation. In vitro experimental results demonstrating inhibition of carboxypeptidase N (kininase I), the inactivator of anaphylatoxin and kinin mediators released in shock reactions, have been advanced as another possible mechanism that may contribute to the spectrum of adverse reactions to protamine.

7.8.1.2 Immune-Mediated Hypersensitivities to Protamine

In what may be the first evidence for an immune-mediated hypersensitivity reaction to protamine, data were presented in 1978 for an anaphylactic reaction mediated by complement-dependent skin-sensitizing IgG antibodies to the protein. Soon after, descriptions of clinical manifestations, temporal details, positive intradermal tests to protamine, known previous exposure to the agent, and immediate responses to IV adrenaline led to a diagnosis of type I anaphylactic responses in three patients following IV administration of protamine sulfate. Published incidences of the risk of immediate adverse reactions to protamine sulfate during cardiopulmonary bypass surgery vary from 0.06 to 10.7 %. For reactions regarded as "clinically significant" rather than "immediate," reported incidences range from 0.1 to 24 % and the incidence of systemic hypotension following protamine administration is said to be 1.76–2.88 %. A significantly higher incidence of anaphylaxis to protamine in insulin-dependent diabetics than in patients not receiving insulin suggested sensitization by protamine in NPH-insulin preparations. In one early review of 1,150 patients given protamine, anaphylactoid reactions occurred in 3 % of diabetic patients but in only 0.2 % of nondiabetics. Other assessments of the incidence of anaphylaxis after protamine reversal of heparin in patients on protamine-insulin therapy range from 0.6 to 27 %. To determine whether anti-protamine IgG and/or IgE antibodies mediated the reactions in protamine-insulin-dependent diabetics, diabetics and nondiabetics who reacted to IV protamine and diabetics who tolerated protamine during surgery were studied. Anti-protamine IgE and IgG antibodies were judged to be significant risks for acute protamine reactions in diabetics who had received protamine-insulin. IgE antibodies were not found in any patient without previous exposure to protamine-insulin. In nondiabetic patients, the presence of anti-protamine IgG antibodies was shown to be a significant risk, but about 30 % of patients who reacted had neither anti-protamine IgG or IgE antibodies. In a follow-up

study of a single patient who had a life-threatening reaction to IV protamine, serum IgE and IgG antibody levels showed a twofold and 70-fold rise, respectively, 1 month after the reaction. Intradermal skin tests with protamine sulfate did not discriminate between the test subject and nine normal subjects who had no previous exposure to protamine and no protamine-reactive antibodies. In vitro challenge of basophils with protamine in histamine release experiments proved inconclusive. While it was clear that subcutaneous injection of protamine in insulin preparations induced protamine-specific antibody responses, it remained important to know whether single-dose IV protamine administration could provoke an antibody response. IgE and IgG antibodies were found in 18 and 16 %, respectively, of previously sero-negative patients 4–6 weeks after a single IV dose of protamine. Appearance of antibodies was associated with insulin-dependent diabetes and male gender. Other possible risk factors for protamine sensitivity include allergy to fish (protamine is obtained from fish testes), men who have undergone vasectomy, and infertile men. Evidence for each of these is not compelling. Fish muscle and skin, not testes, are usually consumed, and in the latter two cases, antibodies to sperm and hence protamine have been suggested to be increased risks, but clear demonstrations of associations have not been forthcoming so far.

7.8.1.3 Diagnostic Tests

Diagnostic tests for hypersensitivity to protamine are problematic. Protamine skin tests have been shown to have poor specificity with irritant responses in normal controls resulting from intradermal injections of concentrations of 100–1,000 μg/ml. The recommended concentration for intradermal testing is 1 μg/ml, although this concentration, and a concentration of 10 μg/ml, has been found to give false-positive results. The uncertainty associated with the specificities of tests for protamine were highlighted in a prospective study of patients undergoing elective cardiac surgery and subjected to skin and serum antibody tests for protamine reactivity. Intradermal tests on 32 patients with protamine 1 μg/ml were positive in four (13 %) despite the absence of a clinical reaction in all of the patients. This 87 % specificity for protamine was almost the same as the 91 % specificity obtained with intradermal saline. At a concentration of 10 μg/ml, intradermal protamine injections were positive in ten (31 %) of the patients. Tests for protamine-reactive IgE and IgG serum antibodies were positive in 46–54 % and 100 % of patients, respectively. These high incidences of false-positive reactions demonstrated the unsuitability of skin and antibody tests for screening patients before administration of protamine.

7.8.2 Aprotinin

Aprotinin, a protease inhibitor isolated from bovine lung, is a single chain polypeptide of 58 amino acid residues, molecular weight 6,512 Da. Marketed as Trasylol (Bayer) and Antagosan (Aventis Pharma), aprotinin inhibits fibrinolysis, reduces thrombin generation, and maintains platelet function, properties that explain its prophylactic intravenous use in cardiac surgery, organ transplantations, and hip surgery where reductions in bleeding, blood loss, and transfusion needs are important. It is also thought that aprotinin may help to reduce inflammatory reactions after cardiopulmonary bypass surgery by interacting with enzymes activated during the surgery. In addition, the polypeptide's antifibrinolytic action has led to its topical application in ready-to-use tissue sealants or so-called fibrin glues to maintain hemostasis. Given its protein nature and bovine origin, aprotinin as a foreign protein can induce an immune response in humans with the formation of specific antibodies, both IgG and IgE, and occasionally anaphylaxis. Previous exposure is considered to be the major risk factor for a severe adverse reaction to the polypeptide, with most reactions generally occurring within some months of initial or previous administration. Other risk factors are said to be the consumption of beef, milk in its different forms, and egg albumin.

7.8.2.1 Adverse Reactions to Aprotinin

According to a 2007 analysis of aprotinin-induced anaphylaxis in over 12,000 patients exposed to the drug in cardiac surgery, the incidences of hypersensitivity reactions were 4.1 %, 1.9 %, and 0.4 % in less than 6 months, 6–12 months, and more than 12 months reexposure intervals, respectively. In addition to anaphylaxis, clinical manifestations of hypersensitivity include bronchospasm and cutaneous symptoms of pruritus, urticaria, and exanthema. Of 124 cases of aprotinin-induced anaphylaxis with 11 deaths identified in the period 1963–2003, the risk of anaphylaxis in previously exposed patients was ~2.8 %. The reexposure interval was <3 months in 72 % (38) of the 53 affected patients. An adverse reaction incidence of 2.8 % (seven patients) was also found in an analysis of 248 reexposures to aprotinin over the period 1988–1995. Five of the seven patients reacted within 3 months of reexposure and two reacted to a loading dose following a test dose. The same outcome was seen in two patients who reacted within 5 min of the loading dose given after a test dose even though the patients had been pretreated with corticosteroids and antihistamines. The safety of the use of aprotinin, and its reuse, in pediatric cardiothoracic surgery was assessed in a retrospective review of 681 first exposures, 150 second exposures, and 34 third or higher exposures to the agent. The incidences of reactions were found to be low—specifically 1 %, 1.3 %, and 2.9 %, respectively, with reactions no more likely to occur in any of the three categories. Skin testing showed a negative predictive value of 99 % and a positive predictive value of 20 %. Aprotinin-reactive IgE antibodies were not detected in seven of eight reactive patients tested, leading to the conclusion that the test "would not be clinically useful." In view of the small number tested and the absence of any details of the test methodology, this conclusion should not yet be taken as final. However, on the basis of the results of this study, the overall conclusion that the use and reuse of aprotinin in children in cardiothoracic surgery is essentially safe and that any reactions that do occur can generally be managed successfully seems sound. Screening of 520 preoperative adult serum samples from cardiosurgical patients for aprotinin-reactive IgG antibodies revealed that of 22 positive sera (4 %), only three were from patients with documented aprotinin preexposure. Only one of the 22 positive sera was also positive for IgE antibodies to aprotinin. This patient experienced anaphylaxis and also had recent IV preexposure to the drug. The investigators concluded that the clinical significance of IgG antibodies to aprotinin is questionable and the presence of the antibodies is not a reliable prediction of previous exposure. What makes the interpretation of the significance of serum antibodies to aprotinin even more difficult is the presence of IgG and IgE antibodies in 55 % of patients with an allergic reaction and 32 % of nonreactors. In addition, half the patients still have serum IgG to aprotinin 4 years after receiving the drug. In a study of anaphylaxis after reexposure to aprotinin during cardiac surgery, the incidence of anaphylaxis was found to be 2.5 % (3 of 121 patients) but detected IgG and IgE anti-aprotinin antibodies were not always clinically relevant. All three anaphylactic patients had high levels of aprotinin-reactive IgG and two had high levels of IgE antibodies. According to the authors, a comparison of the propensity of a patient to react adversely, and the length of the aprotinin exposure—reexposure interval, indicated that within a reexposure interval of 6 months, aprotinin should be used with caution only in exceptional cases such as patients with a high risk of bleeding. Aprotinin in fibrin sealants has also been reported to trigger anaphylaxis. For example in one case, topical reexposures to fibrin sealants containing aprotinin did not provoke adverse reactions, but anaphylaxis did result following IV administration of aprotinin during coronary artery bypass graft despite a negative test dose 5 min before. Postoperative serological tests revealed elevated aprotinin-specific IgG and IgE antibodies.

In 2007, aprotinin was temporarily withdrawn worldwide until results from the Canadian BART (Blood conservation using antifibrinolytics: a randomized trial) study conducted in high-risk cardiac surgery patients was completed and evaluated. Sales of the drug were suspended in May 2008, but this suspension was lifted in Europe by the European Medicines Agency in February 2012.

For skin testing, aprotinin can be used at a concentration of 10,000 IU/ml in prick tests and up to a maximum of 100 IU/ml intradermally.

7.8.3 Latex

Natural rubber latex, from the *Hevea brasiliensis* tree, is used widely in a vast array of rubber goods including, until recently, many medical products. Beginning in the 1980s and extending into the 1990s, the number of reports of allergy to latex, particularly anaphylaxis, increased spectacularly. Two main reasons for this appear to have been increasing concern about transmissible infections like AIDS, leading to a dramatic increase in the use of rubber gloves and the so-called *bias of ascertainment*, that is, the greater ease of recognizing a condition once it has been identified and defined diagnostically. The incidence of allergic sensitivity to latex in the general population is estimated to be about 2.1–3.7 % but can be much higher in risk groups such as dentists (up to 15 %) and spina bifida patients (up to 64 %). The steady increases in the reports of anaphylaxis to latex appear to have peaked presumably because of widespread increased awareness of the problem, more stringent requirements for glove manufacture, the decrease in numbers of latex surgical products, the setting up of "latex-free" medical environments, warning labels, and regulatory requirements. Incidences of anaphylaxis to latex during anesthesia and surgery, estimated to represent about 20 % of all cases, may now vary greatly as shown by the widely contrasting incidences revealed, for example, by recent surveys in France where the figure was 25 times the Australian incidence (see Sect. 7.2 and Table 7.1). A comparison of clinical manifestations of latex anaphylaxis in 1,158 cases not related to anesthesia and surgery with 583 cases occurring during surgery revealed no instances of cardiovascular collapse in the former group but an incidence of 50 % in the latter group (compare Table 7.2). Respiratory symptoms were more evenly distributed, but cutaneous reactions occurred with much higher frequency (98 %) in the non-anesthesia/surgery group. This may be, in part, a reflection of the draping of patients during surgery. In addition, to the well-known at-risk groups of healthcare workers and spina bifida patients, other risk factors include atopy, allergies to fruits, previous exposures such as repeated insertions of latex catheters and indwelling catheters, and patients who have undergone multiple surgical procedures. Pretreatment with antihistamines and corticosteroids is not always effective in preventing anaphylactic reactions to latex so an emphasis should be placed on prevention. This, according to Phil Lieberman, involves the introduction of measures and changes in operating, treatment, and recovery rooms that includes the employment of non-latex or latex-free gloves (most important), catheters, bandages, tapes, tubing, bite blocks, breathing system, and electrocardiogram and pulse oximetry leads. The pharmacy should institute latex-free protocols and the hospital should set up a latex-free cart with non-latex gloves, stethoscope, masks, pressure cuffs, neoprine bags, uncuffed polyvinyl chloride endotracheal tubes, Webril™ tourniquets, and so on.

For the diagnosis of latex allergy, a detailed history, including domestic and occupational history, is an essential and prime requirement. Skin tests with commercially available standardized latex extracts can be used and immunoassays for latex-reactive IgE antibodies are available, for example, the Phadia's ImmunoCAP® assay (Thermo Scientific). Extracts of latex gloves for prick and patch tests are easily prepared by extracting minced material in physiological saline followed by protein determinations and stanardization for IgE antibody binding with a pooled standard serum sample from allergic subjects. Although delayed-type reactions are occasionally seen, most reactions of concern are IgE antibody mediated. *H. brasiliensis* extracts contain at least 13 IgE-binding components that react with sera from latex-allergic subjects. About half of these can be considered major allergens or important for cross-reactivities with, for example, some fruits. An interesting relationship exists between some allergies to natural rubber latex and some fruits. The latex components responsible for this cross-recognition include hevein, a

wound-induced protein from the rubber tree, and a class 1 chitinase (Hev b 11). The major latex allergens identified and purified so far are Hev b 1 (rubber elongation factor), Hev b 3 (small rubber particle protein), and Hev b 4 (a glucosidase), important for reactions in spina bifida patients; Hev b 2 (β-1,3-glucanase) and Hev b 6 (prohevein/hevein), important in latex-fruit cross-reactions; and major allergens Hev b 5 (an acidic protein) and Hev b 13 (a lipolytic esterase).

7.9 Heparin

Heparin is a sulfated glycosaminoglycan polymer of repeating disaccharides most commonly composed of 2-*O*-sulfo-α-L-iduronic acid and 2-deoxy-2-sulfamido-α-d-glucopyranosyl-6-sulfate. Disaccharides containing 3-*O*-sulfated d-glucosamine or d-glucosamine with its free amino group are more rarely found. Preparations can range from unfractionated to low molecular weight (LMW) heparins which include certoparin, dalteparine, enoxaparine, nadroparine, tinzaparine, and reviparine. The polymers used medicinally and supplied commercially generally have molecular weights in the range 12–15 kDa. Heparin is stored in the granules of human mast cells and basophils. It is used as an anticoagulant, preventing but not breaking down clots by binding to antithrombin III and activating the enzyme which then inactivates thrombin and other proteases involved in clotting, particularly factor Xa. It is often administered to patients during cardiac surgery including pulmonary bypass surgery and for acute coronary syndrome, atrial fibrillation, deep vein thrombosis, and pulmonary embolism. Heparin is given parenterally, usually by infusion, since it is not well absorbed from the gut and has a half-life of about 1 h (4–5 h for LMW heparins and 1–2 h for unfractionated heparin).

7.9.1 Adverse Reactions to Heparin

The overall incidence of adverse reactions to heparin has been estimated at 0.2 % with heparin-induced thrombocytopenia, rare cases of anaphylaxis, a few delayed reactions, and some adverse skin reactions making up most of the reports. Heparin can bind to surface-bound platelet factor 4 (PF4) on the platelet surface. IgG antibody formation is common after heparin administration and some antibodies react with the heparin–PF4 complex on the platelets activating the platelets via their Fc pieces and causing the release of microparticles that promotes thrombin formation. A minimum of 12–14 saccharide units of heparin are required to form the heparin–PF4 complex and elicit antibody formation to the complex. Although this reaction is often classified as a type II cytotoxic hypersensitivity response, the formation of immune complexes on the platelet surface also suggests a type III mechanism (see also Sect. 3.6). Up to about 50 % of heparin-treated patients may form antibodies reactive with the heparin–PF4 complex. A few cases of heparin-induced anaphylaxis have been seen in hemodialysis patients. Other recorded immediate reactions include rhinitis, urticaria, pruritus, and bronchospasm. Given the quite marked antigenicity of heparin, the rarity of immediate reactions is somewhat surprising. Delayed hypersensitivity reactions to LMW heparins are occasionally seen with generalized maculopapular rashes, skin necrosis, baboon syndrome, DRESS, and Lyell's syndrome recorded. Cross-reactions between unfractionated and LMW heparins occur and even pentosan polysulfate and danaparoid, both chemically distinct from heparin, may show some cross-reactivity. In one study of delayed heparin allergy, 81 and 45 % of patients showed cross-reactivity to danaparoid and pentosan polysulfate, respectively. In a skin test study of cross-reactivity between unfractionated and LMW heparins in patients with suspected delayed hypersensitivity to heparin, 11 of 15 patients (73 %) showed cross-reactivity between heparins and/or danaparoid, six patients (40 %) reacted to LMW heparins only, and nine patients (60 %) reacted to both the unfractionated and LMW preparations. Danaparoid was tolerated in six of eight patients and hirudin in all three patients tested. Other reported adverse cutaneous reactions to LMW heparins include skin necrosis due

to vasculitis (described as a type III Arthus reaction) and erythematous, well-circumscribed lesions without necrosis, usually secondary to a delayed-type IV reaction.

7.9.2 Diagnostic Methods

Diagnostic skin testing for immediate reactions is undertaken with undiluted commercial heparin preparations as prick test solutions while a starting dilution of 1–1,000 stepping up to 1–10 is employed for intradermal tests. With cross-reactivity and possible alternative drugs in mind, skin tests should include unfractionated and LMW heparins, danaparoid, hirudin, enoxaparin, and perhaps synthetic pentasaccharides such as fondaparinux. In one patient with type I hypersensitivity and a positive skin test to dalteparine, other LMW heparins were also skin test positive, but unfractionated heparins, fondaparinux, and the recombinant hirudin lepirudin were skin test negative and tolerated by the patient. For delayed reactions, prick testing is undertaken with undiluted commercial preparations and for intradermal tests a 1–10 dilution can be used. Readings should be carried out after 30 min, 24, 48, 72, and 96 h or even longer if a very late response is thought to be a possibility. Undiluted and 10 % aqueous solutions may be applied for 24 or 48 h as patch tests and read at 24 or 48, 72, and 96 h and then again at 1 week. Patch tests are said to yield a high rate of negative results so the very late readings (72–96 h and beyond) may prove important.

7.9.3 Danaparoid and Hirudins

Danaparoid is chemically distinct from heparin and generally shows little or no cross-reactivity in heparin-intolerant patients. It is a mixture of heparan sulfate, dermatan sulfate, and chondroitin sulfate and works by inhibiting factor Xa. Danaparoid should be included in tests on patients who cannot tolerate LMW heparins. Reported reactions to the drug include rash, pruritus, and reactions (some delayed) at the injection site. Prick tests are undertaken with undiluted commercial preparations and intradermal tests with dilutions of from 1–1,000 to 1–10. For patch testing, undiluted or 10 % aqueous solutions are applied for 24 or 48 h with readings at 24 or 48, 72, and 96 h and again at 7 days.

Hirudin is a naturally occurring 65 amino acid polypeptide anticoagulant from the salivary glands of the leech used in medicine, *Hirudo medicinalis*. It is the most potent inhibitor of thrombin and has thrombolytic properties, preventing and dissolving clots and thrombi. Desirudin and lepirudin are recombinant forms. Hirudin is a mixture of isoforms of the protein whereas the two recombinant forms are homogeneous preparations. Desirudin differs from hirudin only by the absence of a sulfate group on Tyr-63; lepirudin differs by absence of the same sulfate but also by substitution of a leucine for isoleucine at the N-terminal of hirudin. Being completely different in chemical structure from heparins, there is no cross-reaction between hirudins and heparins. Figures of 0.015 % and 0.16 % have been reported for anaphylaxis on first and subsequent exposures, respectively. Urticaria and angioedema are other reported type I reactions. Injection site reactions may occur and eczematous plaque and granulomatous delayed hypersensitivity reactions have been described. Clinical trials showed allergic reactions to desuridin in 1.6 % of treated patients. Since both recombinant hirudins are prepared in genetically modified yeast cells, allergic reactions may occur in subjects allergic to yeast. Antibodies have been reported in patients treated with hirudins so there is potential for immunological cross-reactivity between the three preparations. Fatal anaphylactic/anaphylactoid reactions have occurred with hirudin therapy. The product information for desirudin (Revasc) states that hirudin-specific IgE evaluations may not be indicative of sensitivity to desirudin since the test was not always positive in the presence of symptoms. Undiluted commercial preparations are employed for prick testing and a dilution of 1–100 for intradermal tests. Pure and 10 % aqueous solutions are used for patch testing with application and reading times as for danaparoid. Despite the statement on Revasc (above), the peptide nature of hirudins makes tests for IgE and IgG antibodies feasible.

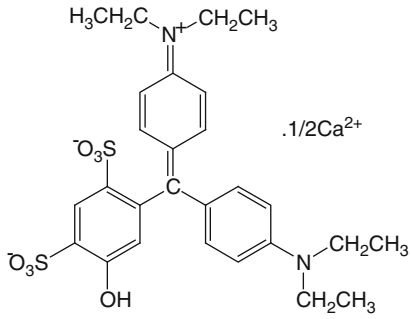
7.9.4 Fondaparinux

Fondaparinux, a synthetic pentasaacharide with structural identity to a sequence of five sugar units of heparin, is a new class of antithrombotic agents that selectively inhibits coagulation factor Xa. Unlike heparin, fondaparinux does not inhibit thrombin and it has proved more effective than enoxaparin in preventing venous thromboembolism after major surgery. Tolerance to fondaparinux in some heparin-sensitized patients has been demonstrated and antibody-induced thrombocytopenia caused by fondaparinux has so far not been reported. Results indicate that fondaparinux has a low allergenic potential with an incidence of allergic skin reactions of only 0.4 %. In one recent prospective study involving 231 patients, no cross allergies were observed in patients with delayed-type hypersensitivity reactions to heparin. The incidence of allergic cutaneous reactions to the drug was one-twentieth of the heparin figure, leading the investigators to conclude that in selected patients, fondaparinux might substantially improve patient care, therapeutic safety, and cost-effectiveness of anticoagulant therapy. Delayed-type reactions to *pentosanpolysulfate* have been reported without exposure to the drug if patients are sensitized to structurally related heparins. In these cases, and when hirudin must be avoided, fondaparinux appears to be a valuable alternative.

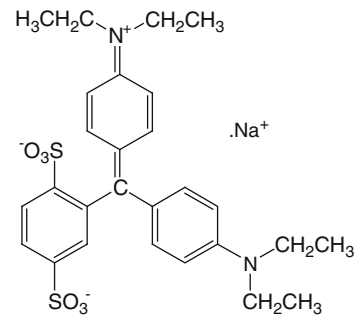
7.10 Patent Blue V, Isosulfan Blue, and Methylene Blue

Water-soluble blue dyes with and without isotope, in particular, patent blue V, isosulfan blue, and methylene blue, are being increasingly used to identify sentinel lymph nodes in melanoma patients and in cases of breast, bladder, cervical, endometrial, and other cancers. Reports of adverse reactions to blue dyes date to at least the 1960s and it is now clear that type I allergic reactions including anaphylaxis occur occasionally with all three of the above-mentioned drugs. Unfortunately, confusion surrounds the terminology used for *patent blue V* and *isosulfan blue*. The chemical structures of the two triarylmethane dyes are shown in Fig. 7.22 where it can be seen that patent blue V, usually obtained as a calcium-chelated dimer or sodium salt, differs from isosulfan blue in the hydroxyl group at position 5 and the sulfonate groups at positions 1 and 4 while isosulfan blue has the sulfonate groups at positions 2 and 5. The literature information on the two dyes is often contradictory, especially in relation to synonyms and sometimes even CAS numbers, but the information summarized in Table 7.13 appears to be correct.

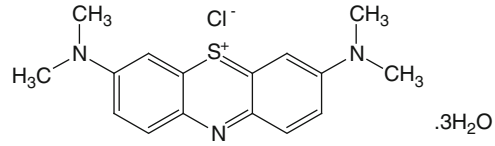
In a recent study of adverse reactions to patent blue V in 7,917 patients with breast carcinoma, patients were given patent blue V and technetium-99 m (^{99m}T) colloid as part of sentinel lymph node biopsy. The study was part of a UK-wide sentinel lymph node biopsy NEW START surgical training program and the Axillary Lymphatic mapping Against Nodal Axillary Clearance (ALMANAC) multicenter trial under the auspices of the Medical Research Council of the UK. Adverse reactions were seen in 72 of the 7,917 (0.91 %) patients and no patients died. Four patients had nonallergic reactions (0.05 %), 23 (0.29 %) experienced minor (grade I) allergic skin reactions, 16 (0.2 %) had grade II reactions, and five (0.06 %) had severe grade III reactions. In 24 (0.3 %) patients the adverse reaction was not further described or graded. By comparison, collective results from American studies in which isosulfan was used for sentinel lymph node biopsies in breast and melanoma patients showed allergic reactions in 119 (1.42 %) of 8,372 patients. With regard to the severe allergic reactions (grade III), the figures were 0.44 % and 0.06 % for isosulfan blue and patent blue V, respectively. Pharmacovigilance files provided by the manufacturer on the involvement of patent blue V in 158 adverse events collected worldwide and from the literature over the 5 year period 2002–2007 revealed two adverse events for every 10,000 patients. Note that biphasic reactions to both dyes have been observed with a second episode occurring 3–8 h after the initial reaction.



Patent blue V



Isosulfan blue



Methylene blue

Fig. 7.22 Chemical structures of dyes used for sentinel lymph node biopsies—the triarylmethane dyes, patent blue V and isosulfan blue, and the thiazine dye, methylene blue. The latter dye is not always approved for sentinel lymph node localization

Table 7.13 Comparison of blue dyes that have been used for sentinel lymph node localization

Dye[a]	Other names	CAS nō[b]/CI nō[c]
Patent blue V[d]	Disulfine blue Acid blue 3 Patent blue violet Food blue 5 Trade name—Bleu Patenté V: Guerbet[e]	3536-49-0/42051
Isosulfan blue	Trade name—Lymphazurin™ [f]	68238-36-8/– [g]
Methylene blue[h]	Methylthioninium chloride Basic blue 9 Swiss blue Aniline violet Solvent blue 8	7220-73-3/52015

[a]See Erratum on nomenclature. J Nucl Med 2003;44:649
[b]CAS nō, Chemical Abstract Service unique numeral identifier number
[c]CI nō, Color Index number
[d]Also used as food colorant with number E131. Banned as a food dye in Australia and USA because of possibility of allergies
[e]25 mg/ml
[f]10 mg/ml
[g]No CI number yet assigned
[h]10 mg/ml injection

For diagnosing immediate hypersensitivity to patent blue V and isosulfan blue, intradermal testing is generally satisfactory using a 1:100 dilution of the stock solution (1 %). Flow cytometric methods using CD63-positive basophils have also been successfully used to demonstrate true type I reactions to patent blue V, but the ImmunoCAP® assay for isosulfan blue and some other immunoassay methods for detecting IgE antibodies to patent blue V have proved negative in the few studies carried out. Immunological cross-reactivity between the two dyes has been demonstrated in both skin and CD63 expression studies and, as reactions frequently occur on what is apparently first exposure, sensitization by exposure to triarylmethane dyes in various products encountered in everyday life is assumed.

Methylene blue, or methylthioninium chloride, is a thiazine dye (Fig. 7.22, Table 7.13) unrelated in structure to patent blue V and isosulfan blue. Although not always approved for the purpose, methylene blue has been used for sentinel lymph node localization. Because it can cause necrosis on subcutaneous injection, it is generally

given IV. In a prospective study of 30 patients in the USA, the dye was used instead of isosulfan blue to localize lymph nodes. It proved successful in 90 % of the cases giving results similar to isosulfan blue. The investigators pointed out the substantial cheaper cost of methylene blue.

Summary

- NMBDs are responsible for ~60 % of anaphylactic episodes in the perioperative period.
- Succinylcholine accounts for about one-third of the reactions. Reactions to rocuronium are significantly higher in Europe than in Australia.
- Cardiovascular reactions are common and serious symptoms during anaphylaxis to NMBDs. Reactions may progress to cardiovascular collapse in up to 80 % of cases and may be the only symptom in 60 % of cases. Bronchospasm is seen more often in anaphylactic than in anaphylactoid reactions to NMBDs.
- Incidences of anaphylaxis to NMBDs are 1 in 5,500 in France, 1 in 5,200 in Norway, and 1 in 10,000 in Australia.
- Reactions to NMBDs are mediated by IgE antibodies with specificity for tertiary and quaternary ammonium ions, but adjoining structures may also be recognized. Recognition of the substituted ammonium groups accounts for the extensive cross-reactivity between the NMBDs.
- Substituted ammonium groups occur widely in many drugs and chemicals which means that NMBD-reactive IgE antibodies cross-react with many other drugs. Reaction with morphine is particularly pronounced.
- Considering fine structural recognition, the combining site specificities of NMBD-reactive IgE antibodies fall into five main groups.
- Diagnosis of reactions is effected by skin testing with free drugs, IgE antibody assays, and the tryptase assay. Because of morphine's striking capacity to detect and cross-react with NMBD-reactive antibodies in patients' sera, it is used in solid phase form to detect NMBD-reactive IgE antibodies. Solid phase complexes of NMBDs and some analogs (such as choline for succinylcholine) have also been used to detect IgE.
- When used as a diagnostic aid for allergy to NMBDs, the BAT has been found to be specific but disappointingly lacking in sensitivity.
- Reversal of rocuronium-induced NM block with the cyclodextrin sugammadex has highlighted the question of changed allergenicity of such chemically sequestered drugs in host–guest complexes.
- With at least seven current reports in six different countries of the mitigation of rocuronium-induced anaphylaxis by sugammadex, this specifically modified cyclodextrin might prove to be a new and useful treatment to manage rocuronium-induced anaphylaxis.
- Many patients who experience anaphylaxis to an NMBD do so on first exposure, raising the question of the sensitizing source. Pholcodine, which cross-reacts with NMBDs, has been suggested to be this source and this appears to be supported by a large boost in IgE antibodies to pholcodine, morphine, and succinylcholine following dosage with a cough syrup containing pholcodine.
- "Natural" IgE antibodies to phosphorylcholine have been suggested as an alternative explanation for preexisting NMBD allergic sensitivity.
- Anaphylactic reactions occasionally occur to the hypnotics thiopentone and propofol. Both are diagnosed by skin testing and the former also by a specific IgE test.
- The colloid hydroxyethyl starch is well tolerated with an incidence of adverse events less than gelatin and dextran. The incidence of allergic reactions is 0.0004 % and the risk of life-threatening reactions 0.006–0.085 %.
- Gelatin, marketed as Haemaccel® and Gelofusine®, can cause both IgE- and non-IgE-mediated reactions. Clinical manifestations include anaphylaxis, urticaria, bronchospasm, and sneezing.
- Pre-injection of small MW dextran 1 reduces the incidence of dextran-induced anaphylaxis from 25 to 3 per 100,000.

- True IgE-mediated reactions to local anesthetics are extremely rare; many reactions appear to be vasovagal responses but delayed reactions are well known.
- Anaphylactic reactions to protamine and aprotinin occur infrequently and anaphylaxis to latex has decreased markedly in recent years.
- The overall incidence of adverse reactions to heparin has been estimated at 0.2 % with heparin-induced thrombocytopenia, rare cases of anaphylaxis, a few delayed reactions, and some adverse skin reactions making up most of the reports.
- Danaparoid and hirudins are chemically distinct from heparin and generally show little or no cross-reactivity in heparin-intolerant patients. Reported reactions to danaparoid include rash, pruritus, and reactions (some delayed) at the injection site. Figures of 0.015 and 0.16 % have been reported for anaphylaxis to hirudins on first and subsequent exposures, respectively. Urticaria and angioedema are other reported type I reactions.
- Fondaparinux selectively inhibits coagulation factor Xa and has a low allergenic potential.
- In a multicenter trial on patent blue V, adverse reactions occurred in 0.91 % of patients and no patients died. Nonallergic reactions occurred in 0.05 % of patients, 0.29 % experienced minor (grade I) allergic skin reactions, 0.2 % had grade II reactions, and 0.06 % had severe grade III reactions.
- Studies in which isosulfan was used for sentinel lymph node biopsies in breast and melanoma patients showed allergic reactions in 1.42 % of patients. With regard to the severe allergic reactions (grade III), the figures were 0.44 % and 0.06 % for isosulfan blue and patent blue V, respectively.

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Opioid Analgesic Drugs

8

Abstract

Opioid analgesics are one of the most commonly administered groups of drugs in hospitals. These drugs show common structural features, bind specifically to opioid receptors and possess morphine-like pharmacologic action. Tramadol differs from other opioid analgesics in its monoaminergic activity as well as its affinity for the μ opioid receptor. Many opioids are potent histamine releasers producing hemodynamic changes and anaphylactoid reactions, but there seems to be no direct relationship between the histamine plasma concentrations and these changes. True IgE antibody-mediated immediate allergic reactions to opioids are uncommon, although some anaphylactoid reactions are interpreted as allergic, emphasizing the need to investigate whether or not reactions have an immune basis. The histamine-releasing properties of opioid drugs sometimes hamper skin testing, and general unavailability of specific IgE antibody tests contributes to the failure to investigate reactions. Reactions to tramadol, whether anaphylactoid or IgE antibody-mediated, are rare, and the drug is generally considered to be safe with a low potential for adverse reactions. Clinical implications for the diagnosis of opioid drug-induced anaphylactoid and anaphylactic reactions are discussed.

Opioid analgesic drugs (OADs), particularly fentanyl and its analogs alfentanil, remifentanil, and sufentanil, are extensively used for anesthesia and analgesia. Administration of intravenous hypnotics and OADs as a high dose opioid, low dose hypnotic (usually a benzodiazepine such as midazolam) has found wide application in cardiac anesthesia and to produce procedural sedation for endoscopy, catheterizations, and a variety of other surgical procedures where sedation and pain relief are required. OADs might, therefore, be considered along with other agents used in general anesthesia (Chap. 7), but their general usefulness as analgesics apart from their application in the operating room together with their histamine releasing properties and the large number of members of the opioid family of drugs, both naturally occurring and synthetic, make these drugs worthy of closer and more individual examination.

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_8, © Springer Science+Business Media, LLC 2013

8.1 Terminology

The naturally occurring analgesic drug morphine along with a number of structurally related alkaloids including codeine, noscapine (narcotine), thebaine, and papaverine, and together with about 25 other minor alkaloids of unknown or little-or-no useful pharmacological action, are obtained from the latex from immature seed pods of the opium poppy plant *Papaver somniferum*. The medically important morphine and codeine, and the semisynthetic derivatives such as heroin (diamorphine), hydromorphone, oxymorphone, hydrocodone, and oxycodone, prepared from some of the natural alkaloids (mainly morphine, codeine, and thebaine), were originally designated *opiates*, a generic term used for both natural and synthetic drugs with morphine-like actions. The term *narcotic*, derived from the Greek "narko," (from narkoun, to numb; narkē, numbness) was originally applied to any substance that relieved pain, dulled the senses, or induced sleep. The definition covered substances that produced at least some morphine-like actions including unwanted side effects such as drowsiness, euphoria, nausea, constipation, and dependence and, therefore, also encompassed some substances not necessarily derived from the opium poppy such as cocaine and coca leaves (in the USA at least). The term now carries with it negative connotations since in a legal context, almost universally in the media, and to the layman, it indicates a prohibited drug such as morphine, heroin, or the potent oxy- and hydro-derivatives of morphine and codeine. Therefore, although the terms *opiate* and *narcotic* are still often used, they are no longer useful in a pharmacological context.

Nalorphine (*N*-allylnormorphine), synthesized in 1942, proved to have not only some analgesic action but also strong antagonistic effects on many of the actions of morphine. It was becoming clear that opioid agonists, antagonists (such as naloxone), and mixed agonist–antagonists must act on multiple receptors and by the early 1970s, binding studies with radiolabeled drugs revealed stereospecific opioid binding sites in the central nervous system. Confirmation of the existence of specific receptors for opium alkaloids and related synthetic drugs led to the identification of endogenous peptide ligands, in particular, enkephalins, β-endorphin, and dynorphins for the receptors. These peptides are called opioid peptides since they have effects resembling opiate drugs and, correspondingly, the complementary receptors are called "opioid." The term "opioid drug" then includes naturally occurring and semi- and fully synthetic drugs that produce their effects by binding specifically to any of several different opioid receptors and which are competitively antagonized by naloxone. Not all opioid drugs show a receptor recognition pattern identical to morphine and hence opioid drugs may, or may not, have similar pharmacological actions as the prototypic opioid.

Opioid receptors do not form a homogeneous population and occur in two major branches. The receptors named mu (μ), kappa (κ), and delta (δ), where naloxone acts as an antagonist, forms the main branch while the other branch comprises the nociceptin or ORL_1 receptor which shows no recognition of naloxone. In 1999 an International Union of Basic and clinical Pharmacology (IUPHAR) opioid receptor subcommittee of the Receptor Nomenclature and Drug Classification Committee (NC-IUPHAR) recommended that: "The well-established Greek terminology for opioid receptor types using the descriptors μ (mu), δ (delta) or κ (kappa), is recommended, but the receptor type should be additionally defined as MOP, DOP, KOP, or NOP when first mentioned in publication."

Opioid drugs and peptides that bind to the opioid receptors produce the primary general and specific clinical effects summarized in Table 8.1. The opioids relevant to this review are those that are used in medicine today meaning, therefore, the frequently administered analgesics, particularly morphine, codeine, and synthetic analogs such as fentanyl, meperidine, and methadone.

8.2 Structure–Activity Relationships

Morphine, the prototypic opioid, is a phenanthrene alkaloid with a benzylisoquinoline backbone. It is composed of five fused rings A–E,

Table 8.1 Clinical effects resulting from some opioid ligands binding specifically to opioid receptors

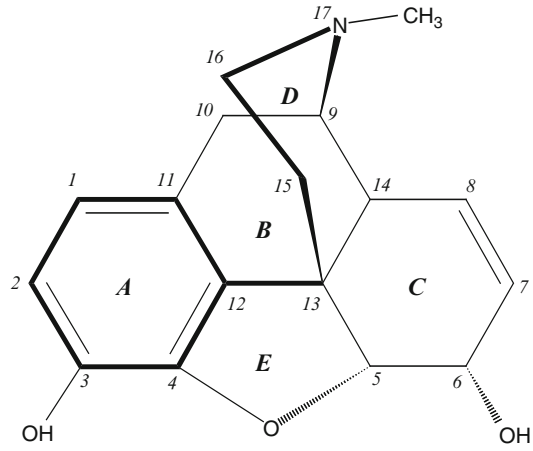
Receptors	Examples of some drugs and endogenous peptides showing preferential binding	Primary general effect	Clinical effects
μ	Morphine Heroin Oxymorphone Hydrocodone Fentanyl, etc.[a] Endomorphins	Central depression	Analgesia; euphoria; respiratory depression; bradycardia; hypothermia; constipation; physical dependence
κ	Nalbuphine[b] Butorphanol Pentazocine Dynorphins	Sedation	Sedation; analgesia; dysphoria; dissociative, deliriant and hallucinogenic effects
δ	Leu-enkephalin Met-enkephalin	Analgesia	Regulation of analgesia and behavior; antidepressant; endocrine function

Some data from Freye E. Opioids in Medicine. Dordrecht: Springer; 2008
[a]Also alfentanil, remifentanil, sufentanil
[b]Drugs that show preferential binding to κ receptors show lower abuse potential

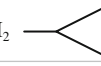
three of which are in approximately the same plane (rings A, B, and E) and with the piperidine ring (D) at right angles (Table 8.2). The naturally occurring levorotatory isomer is the active form; the dextrorotatory isomer is devoid of opioid activities. For morphine and other closely related rigid structures, three structural features in particular are important for analgesic activity, that is, for binding to opioid receptors. They are: a tertiary amine that is part of a piperidine ring; an aromatic ring (A) axially connected to the piperidine ring at the carbon para to the nitrogen (C12–C13 morphine structure Table 8.2) thereby maintaining a fixed geometry between the aromatic ring and the elevated piperidine ring; and polar groups containing an oxygen at C3 of the phenyl ring. Minor changes in the structure of morphine usually cause changes, not only in the physicochemical and pharmacokinetic properties but also in the selectivity profile for opioid receptors. Ring A and the protonated tertiary nitrogen of ring D at physiological pH are two dominant structural features of μ opioid receptor agonists. Changes in the groups attached to nitrogen and at the 3 and 6 positions can produce marked changes in both pharmacological action and potency. For example, increasing the length of the carbon chain attached to the nitrogen from one (methyl) to three to five, especially with unsaturated bonds, produces compounds such as naloxone and naltrexone with high affinity for μ receptors but with antagonistic not agonistic properties. Substitution of a methoxy at position 3, or acetyl groups at positions 3 and 6 instead of hydroxyls, produces the weak μ receptor agonists codeine and heroin, respectively. Changes in the C ring can also lead to compounds of increased analgesic potency as demonstrated with hydromorphone where a 6-keto group replaces the hydroxyl and a 7–8 dihydro linkage replaces the double bond found in morphine. Addition of a hydroxyl group at position 14 generally enhances μ receptor agonist activity. Oxymorphone and oxycodone are examples, both being equal to, or more potent than, morphine. Nalbuphine, also with a 14-hydroxyl group but with an *N*-cyclobutylmethyl instead of an *N*-methyl substituent, is agonistic at the κ receptor and antagonistic at the μ receptor.

A quick perusal of the two-dimensional structures of a range of important opioid drugs reveals that while many of the clinically relevant compounds show clear structural similarities to morphine (Table 8.2), such similarities are not immediately obvious with some other widely used drugs such as the synthesized, and structurally flexible, opioids meperidine (pethidine),

Table 8.2 Structure of morphine and some chemically related naturally occurring or semisynthetic clinically important opioid drugs



Morphine

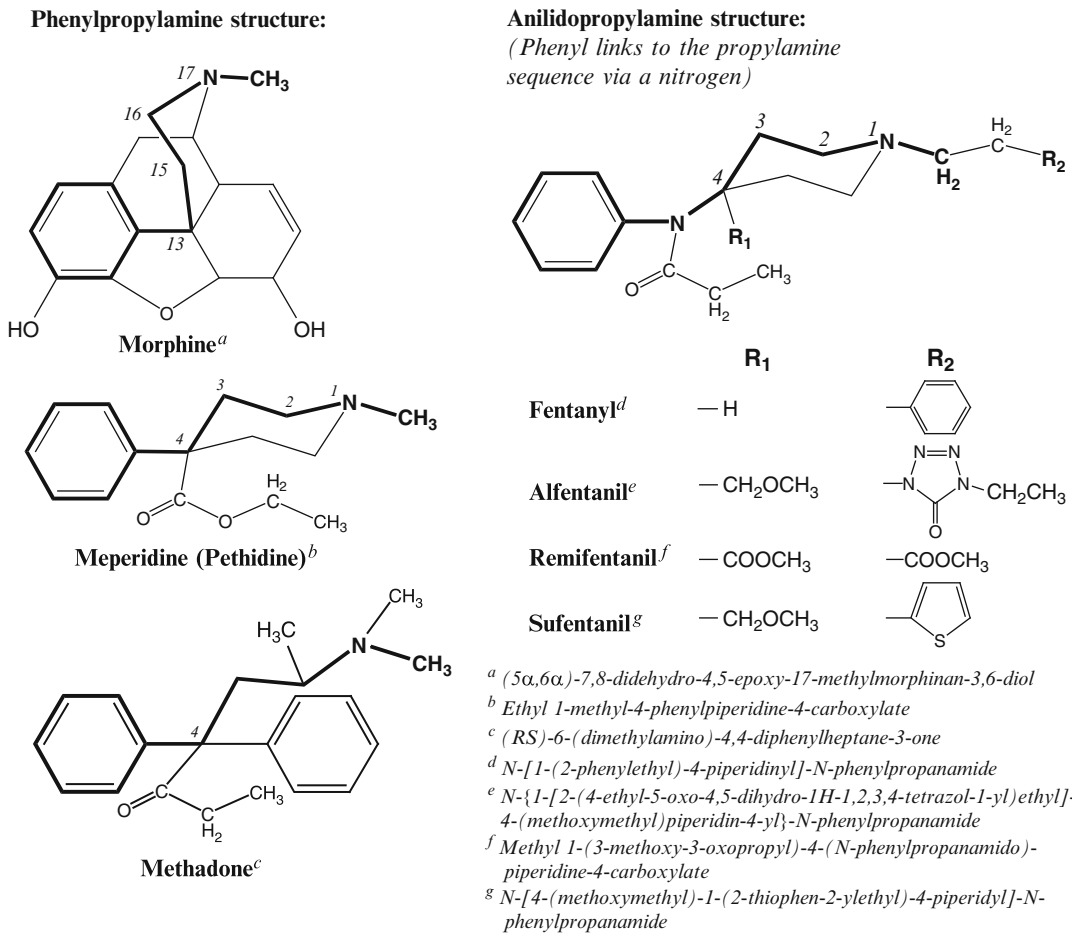
Drug	Substituent at position 3	Substituent at position 6	Substituent at position 14	Substituent at position 17	Bond(s) at positions 7–8
Morphine	–OH	–OH	–H	$-CH_3$	Double
Codeine	$-OCH_3$	–OH	–H	$-CH_3$	Double
Heroin	$-OCOCH_3$	$-OCOCH_3$	–H	$-CH_3$	Double
Hydromorphone	–OH	=O	–H	$-CH_3$	Single
Oxymorphone	–OH	=O	–OH	$-CH_3$	Single
Hydrocodone	$-OCH_3$	=O	–H	$-CH_3$	Single
Oxycodone	$-OCH_3$	=O	–OH	$-CH_3$	Single
Buprenorphine[a]	–OH	$-OCH_3$[b]	[b]	$-CH_2$ 	Single
Naloxone	–OH	=O	–OH	$-CH_2CH{=}CH_3$	Single

From Baldo BA, Pham NH. Histamine-releasing and allergenic properties of opioid analgesic drugs: Resolving the two. Anaesth Intensive Care. 2012;40:216. Reproduced with permission from Australian Society of Anaesthetists

[a]Has a 1-hydroxy-1,2,2-trimethylpropyl substituent at C-7

[b]Endo-ethano bridge between C-6 and C-14

methadone, and fentanyl that also act at the μ opioid receptors like the rigid morphine-like structures. With meperidine, for example, conformational studies have shown that the aromatic ring can be axial or equatorial to the piperidine ring, there is free rotation between them, and the meperidine structure can be fairly well superimposed on the equivalent structural features of morphine (Table 8.2; Fig. 8.1). Both morphine and meperidine have a phenylpiperidine substituent, a sequence of three carbons linked to an aromatic ring via the central carbon C13 in morphine and C4 in meperidine and terminating in an *N*-methyl group. Viewed in isolation, this phenylpropylamine group is seen in morphine in the sequence of positions 13, 15, 16, and 17 attached to the aromatic ring A at C12. The corresponding sequences in meperidine are positions 4, 3, 2, and 1 attached to the aromatic ring. These phenylpropylamine groups are highlighted on the structure of morphine in Table 8.2 and the meperidine structure (Fig. 8.1). Fentanyl has a 4-anilidopiperidine



[a] (5α,6α)-7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol
[b] Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate
[c] (RS)-6-(dimethylamino)-4,4-diphenylheptane-3-one
[d] N-[1-(2-phenylethyl)-4-piperidinyl]-N-phenylpropanamide
[e] N-{1-[2-(4-ethyl-5-oxo-4,5-dihydro-1H-1,2,3,4-tetrazol-1-yl)ethyl]-4-(methoxymethyl)piperidin-4-yl}-N-phenylpropanamide
[f] Methyl 1-(3-methoxy-3-oxopropyl)-4-(N-phenylpropanamido)-piperidine-4-carboxylate
[g] N-[4-(methoxymethyl)-1-(2-thiophen-2-ylethyl)-4-piperidyl]-N-phenylpropanamide

Fig. 8.1 Comparison of the structures of morphine and some important synthetic opioid analgesics. While clear similarities in structure are not always obvious, both morphine and meperidine have a phenylpiperidine structure and a phenylpropylamine grouping, highlighted here as a sequence of three carbons linked to the aromatic ring via the central C13 carbon in morphine and C4 in meperidine and terminating in an *N*-methyl group. Fentanyl, alfentanil, remifentanil, and sufentanil contain a 4-anilidophenylpiperidine and a 4-anilidophenylpropylamine (*highlighted*) structure, but their conformational similarities to morphine and meperidine are readily apparent. Although methadone lacks a piperidine ring, it retains a phenylpropylamine substituent (*highlighted*), and it is thought that the enol tautomer of l-methadone may form a morphine-like conformer by intramolecular hydrogen bonding with the nitrogen (see Figs. 8.2 and 8.3). From Baldo BA, Pham NH. Histamine-releasing and allergenic properties of opioid analgesic drugs: Resolving the two. Anaesth Intensive Care. 2012;40:216. Reproduced with permission from Australian Society of Anaesthetists

rather than a 4-phenylpiperidine sequence but, again, its conformational similarity to meperidine and hence morphine is readily seen although linkage of the aromatic ring to the propylamine sequence is via a nitrogen (Fig. 8.1). Methadone is similar to morphine in receptor binding and pharmacological action; however, the similarity is not, at first sight, readily reflected in structural conformations of the two compounds. Although a piperidine ring is no longer present, like both morphine and meperidine, methadone retains a phenylpropylamine structure (highlighted, Fig. 8.1) and has 11 degrees of rotational freedom. The molecule has a heptane backbone and can be viewed as a central carbon (C4) linked to two phenyl groups, a ketone and a propyl group with an attached acyclic tertiary amine. A number of morphine-like conformers of this structure have been advanced usually based on intramolecular interactions between the carbonyl and amine groups. In

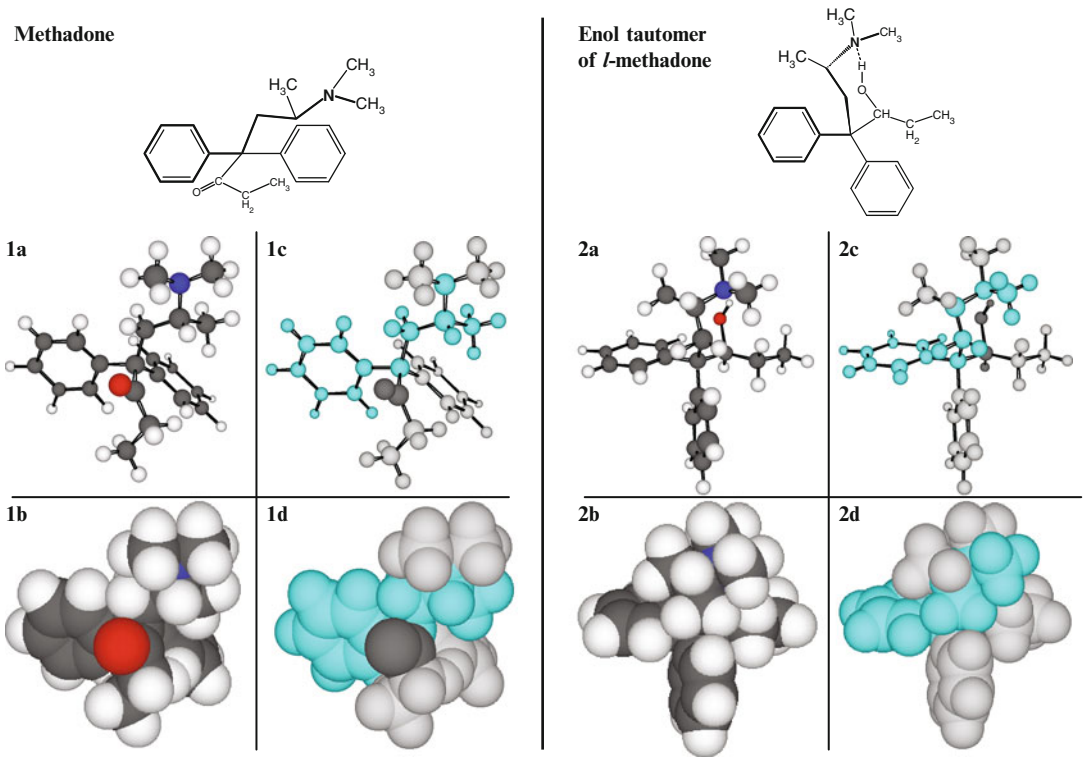


Fig. 8.2 Two-dimensional structures of l-methadone and the enol tautomer of l-methadone together with three-dimensional space-filling ball-and-stick (1a, c and 2a, c) and CPK models (1b, d and 2b, d) for l-methadone and its enol tautomer, respectively. The models of l-methadone show one of many possible conformations while the models of the enol form show the molecule locked by the intramolecular hydrogen bond between the positive charge of the hydroxyl hydrogen and the unshared electrons of nitrogen to give a seven-membered ring, which may be seen as the counterpart of the morphine piperidine ring. In 1c, d and 2c, d the morphine pharmacophore of a tertiary alkylamine at least three atoms away from, and including, an aromatic ring is shown in *blue* while the rest of the molecule is shown in *light gray*

one proposed conformer a pseudopiperidine ring is formed by hydrogen bonding between one of the methyl groups on the nitrogen and the carbonyl oxygen. In another suggested conformer, the enol tautomer of l-methadone is thought to form an intramolecular hydrogen bond between the nitrogen proton and the oxygen thus producing a seven-membered ring that is seen as the counterpart of the piperidine ring of morphine. Models of l-methadone (Fig. 8.2, 1a–d) and its enol tautomer (Fig. 8.2, 2a–d) reveal clear differences in shape and orientation of structures within the methadone molecule. Figure 8.2, 1a–d shows one of many possible conformations due to free rotation about the sp^3-hybridized central C-4 atom of methadone with its tetrahedral symmetry. In the enol form (Fig. 8.2, 2a–d), however, the molecule is locked by the intramolecular hydrogen bond, and the clear difference in the position and orientation of the morphine pharmacophore between the two conformations is clearly visible. Figure 8.3, 2a–d reveals the close similarity between the conformation of the phenylpropylamine sequence in the enol tautomer of l-methadone and the same sequence in selected conformations of morphine (Fig. 8.3, 1a–d) and meperidine (Fig. 8.3, 3a–d) and the anilidopropylamine sequence of fentanyl (Fig. 8.3, 4a–d). It should be remembered though that no conclusive evidence for the existence, one way or the other, of the postulated conformers has been forthcoming and the actual conformation(s) of methadone at the opioid receptor sites is yet to be firmly established.

The synthetic, centrally acting analgesic tramadol with its phenylpropylamine sequence (highlighted in Fig. 8.4a, b) shows some structural

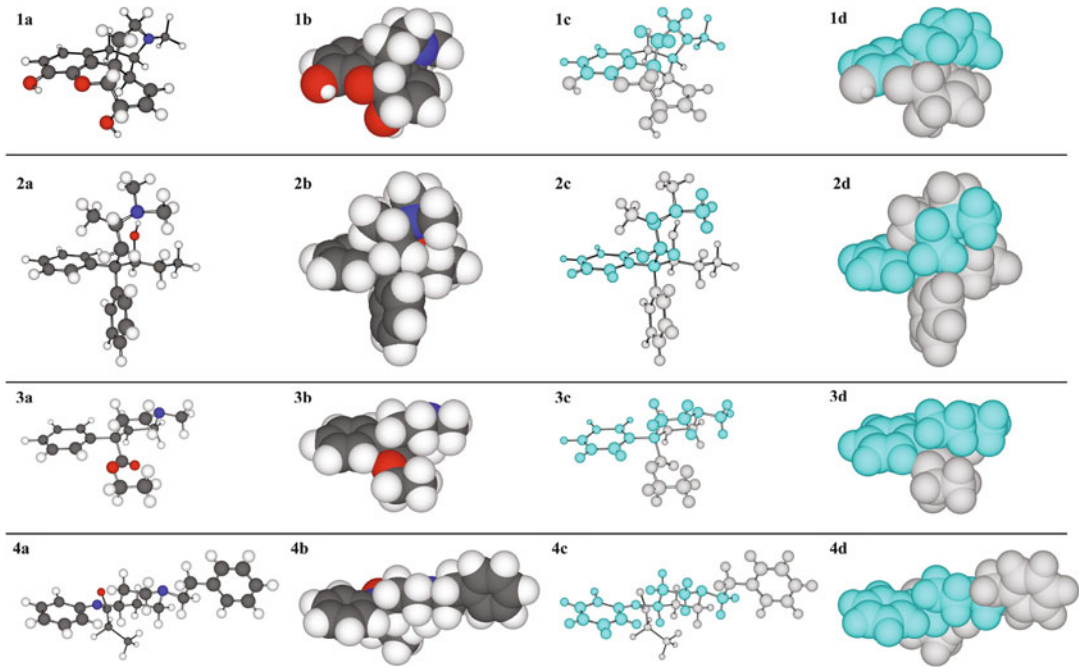


Fig. 8.3 Space-filling ball-and-stick (1a-4a, 1c-4c) and CPK (1b-4b, 1d-4d) three-dimensional molecular models of the structures of morphine (1a-1d), methadone (2a-2d), meperidine (3a-3d), and fentanyl (4a-4d). (For two-dimensional structures of morphine, methadone, meperidine, and fentanyl refer to Fig. 8.1). Conventional colors used for different atoms are shown in structures 1a–4a and 1b–4b. In structures 1c–4c and 1d–4d the morphine pharmacophore of a tertiary alkylamine at least three atoms away from, and including, an aromatic ring is shown in *blue* while the rest of the molecule is shown in *light gray*. For methadone, the seven-membered ring, predicted to form by intramolecular H-bonding of the enol tautomer form with the nitrogen, is shown most clearly in 2a and 2c. This "virtual" ring is seen as the counterpart of the piperidine ring in morphine, meperidine, and fentanyl. Conformations were selected to show the clear similarities in shape, size, and orientation of the pharmacophore in all four drugs. From Baldo BA, Pham NH. Histamine-releasing and allergenic properties of opioid analgesic drugs: Resolving the two. Anaesth Intensive Care. 2012;40:216. Reproduced with permission from Australian Society of Anaesthetists

resemblances to codeine and morphine and is classed as an opioid despite showing some significant differences to the other opioid analgesics. Tramadol exists as four stereoisomers, but the marketed compound is a racemic mixture of the (1R,2R) or (+)-enantiomer and the (1S,2S) or (−)-enantiomer. The drug has a low affinity for the μ opioid receptor, but it can be considered to be a prodrug with the active metabolite *O*-desmethyltramadol (Fig. 8.4c, d), formed by demethylation of the methoxyphenyl group, being a more potent μ receptor agonist than the parent. Only partial inhibition of tramadol's analgesic action by the opioid antagonist naloxone suggested the involvement of a second mechanism of action, and this was explained by the demonstration of monoaminergic activity with effects on serotonin (5-hydroxytryptamine) and norepinephrine (noradrenaline) uptakes. (+)-Tramadol is approximately four times as potent as (−)-tramadol in inhibiting serotonin reuptake while (−)-tramadol is about ten times stronger than the (+)-enantiomer in inhibiting norepinephrine reuptake. These actions are complementary and synergistic and together with the receptor agonist activity lead to inhibitory effects on pain transmission in the spinal cord.

8.3 Classification of Opioid Drugs

Opioid drugs can be classified in a number of ways but, from the medical point of view, the most obvious, simple, and useful classification is on the basis of their clinical importance. To this

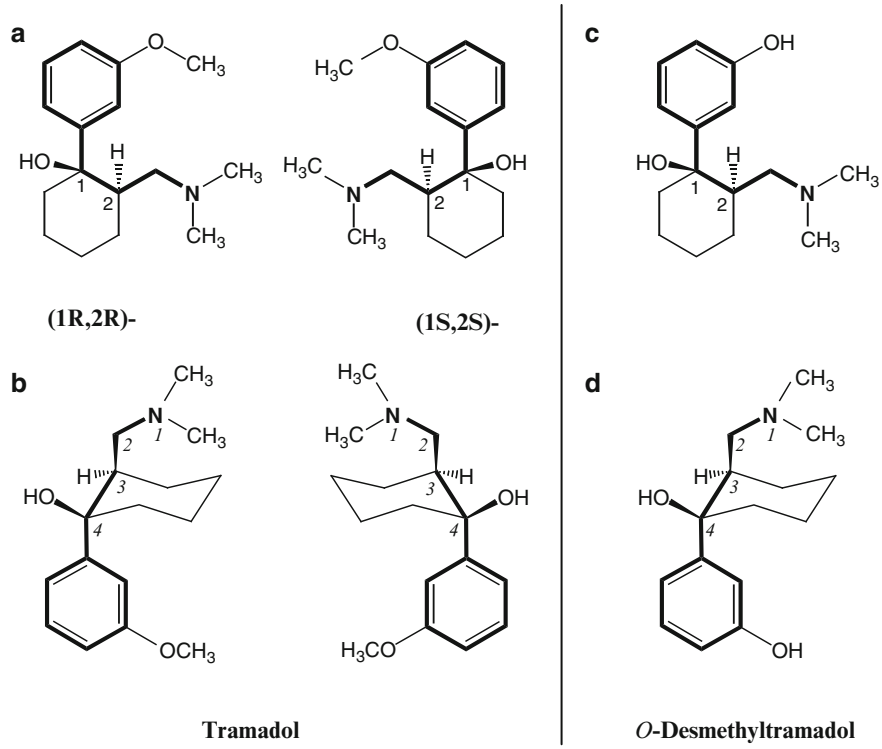


Fig. 8.4 Tramadol, 2-(dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexanol (**a**, **b**), can exist in four different stereoisomeric forms, (1R,2R), (1S,2S), (1R,2S), and (1S,2R), but the drug used (as the hydrochloride) is a racemic mixture of the first two of these isomers that are shown here. Like methadone, tramadol lacks a piperidine ring but like morphine, codeine, meperidine, and methadone it retains a phenylpropylamine structure (see Fig. 8.1), that is, a sequence of three carbons linked to the aromatic ring and terminating in a methyl ammonium group (marked in bold in a and drawn in b to show similarities with structures shown in Fig. 8.1). *O*-Desmethyltramadol (**c**, **d**), the pharmacologically active metabolite of tramadol, has a hydroxyl instead of the methoxy group at position 3 on the phenyl ring

initial classification one can add the origin of a drug, namely, whether it is naturally occurring, semisynthetic, that is, synthesized from a natural product, or prepared by total synthesis. The chemical structure of the compound provides a further useful classification as does the traditional way of comparing the drug on the basis of analgesic potency. Finally, individual drug selectivities for receptors, that is, agonist or antagonist actions at the different opioid receptors, add perhaps the most important information that leads to an understanding of action and hence clinical applications of the various opioid drugs.

Opioid drugs show a wide range of agonist and antagonist effects. For example, morphine is an agonist but in relative terms, phenazocine is classed as a strong agonist and methadone a weak agonist. Naloxone is an antagonist while pentazocine is a mixed agonist–antagonist and nalorphine is classed as an antagonist with a little agonist activity. Most of the opioids used clinically, like morphine, are relatively selective for μ receptors but drugs may interact with additional receptors if given at higher dosages. This can lead to changes in a drug's pharmacological profile. Even at normal clinical doses, agonist–antagonist opioids in particular may recognize more than one receptor, acting as an agonist at one and an antagonist at the other. Table 8.3 summarizes side-by-side the above-listed different classification categories for 13 different clinically relevant opioids.

Table 8.3 Classification of some clinically important opioid drugs

Drug	Origin	Chemical class[a]	Analgesic potency	Main receptor(s) recognized[b]	Function at receptor[b]
Morphine	Naturally occurring	Phenanthrene	Intermediate–strong	μ	Agonist
Codeine	Naturally occurring	Phenanthrene	Weak–intermediate[c]	μ, δ	Weak agonist
Heroin[d]	Semisynthetic	Phenanthrene	Strong	μ	Agonist
Hydromorphone	Semisynthetic	Phenanthrene	Strong	μ	Agonist
Oxymorphone	Semisynthetic	Phenanthrene	Strong	μ	Agonist
Hydrocodone	Semisynthetic	Phenanthrene	Intermediate–strong	μ	Agonist
Oxycodone	Semisynthetic	Phenanthrene	Intermediate	μ	Agonist
Buprenorphine	Semisynthetic	Phenanthrene	Intermediate	μ, δ, ORL_1[e]	Partial agonist
Naloxone	Semisynthetic	Phenanthrene	–[f]	μ, δ, κ[g]	Antagonist
Meperidine (pethidine)	Synthetic	Phenylpiperidine	Weak–intermediate	μ	Weak agonist
Methadone	Synthetic	Phenylpropylamine[h]	Intermediate	μ	Agonist
Fentanyl[i]	Synthetic	Anilidopiperidine	Strong	μ	Agonist
Tramadol[j]	Synthetic	Phenylpropylamine	Weak–intermediate	μ	Weak agonist

[a]Some fit into more than one chemical class
[b]May have weak agonist/antagonist action at other opioid receptors
[c]Acts after metabolized to 6-glucuronide derivative
[d]Metabolized to morphine
[e]Agonist at μ and ORL_1, and antagonist at κ
[f]Antagonist action of opioid analgesics
[g]Highest affinity at μ receptor, low affinity at κ
[h]Also classified as a phenylheptylamine
[i]Similar properties for alfentanil, remifentanil, and sufentanil
[j]Also has monoaminergic activity and metabolized to *O*-desmethyltramadol which is more active at μ receptor than parent drug

8.4 Opioids and Histamine Release

Many opioid drugs are potent releasers of histamine from animal tissues producing, for example, rash, flushing, urticaria, pruritus, hypotension, and mucous. This property continues to cause difficulties and confusion in diagnosis and treatment of various conditions ranging from a simple itch to life-threatening anaphylaxis since many of the symptoms elicited by histamine are similar to those of antibody-mediated type I allergic hypersensitivity. With the view of distinguishing anaphylactoid from true anaphylactic reactions, the histamine releasing properties of those opioid analgesic drugs (OADs) so far studied for this effect will, therefore, be covered in some detail and the findings compared with features of opioid drug-induced immediate IgE-antibody mediated allergic reactions.

8.4.1 Histamine Receptors

Refer to Sect. 3.2.5.1 for an extended discussion of histamine and its receptors. The range of different biological effects displayed by histamine suggested that some of them must proceed via different receptors and application of selected specific agonists and antagonists ultimately revealed three G protein-coupled receptors designated H_1, H_2, and H_3. Each receptor mediates different effects, for example, smooth muscle contraction and some allergic effects including vasodilation and bronchoconstriction proceed under H_1 receptor control; H_2 receptors regulate gastric acid secretion and smooth muscle relaxation and inhibit some immunological cellular processes; and H_3 receptors, first cloned in 1999, control histamine turnover, act as receptors in histaminergic neurons, and inhibit the release of

a number of neurotransmitters including acetylcholine, dopamine, GABA, norepinephrine, and serotonin. Even with this variety of effects, not all of histamine's actions could be accounted for by the three known receptors and, in 2000, a fourth receptor H_4 was cloned and characterized.

In humans, mast cells, and basophils, enterochromaffin-like cells of the gut and histaminergic nerves in the brain constitute the three main sources of histamine. H_1 and H_2 receptors are widely expressed in the body, H_3 receptors are found in the peripheral and central nervous systems, and H_4 receptors in bone marrow and white blood cells as well as in the small intestine, colon, and in organs such as the lung, spleen, liver, and tonsils. Histamine is, of course, a key mediator in allergic processes and, of all the body tissues that show some connection to this prime mediator, the mast cell is probably of greatest interest to allergists and dermatologists. Perhaps surprisingly, expression of histamine receptors on mast cells had been difficult to show, the effect of histamine on mast cells remained unclear, and, intriguingly, effects on cells by some antihistamines did not appear to be mediated by H_1 receptors. The relationship between histamine and the mast cell became a little clearer with the finding that the autacoid induces chemotaxis of mouse mast cells without affecting degranulation of the cells, and this affect can be blocked by a dual H_3/H_4 antagonist but not by H_1 or H_2 antagonists. The chemotactic response, as well as calcium mobilization, is mediated by H_4 but not H_3 receptors with the former, but not the latter, being expressed on the mast cells. In fact, histamine does not seem to have any effect on degranulation of mast cells either alone or in combination with anti-IgE complexes. H_2 and H_4 receptors have been shown to be expressed on human skin and leukemic mast cells, and evidence is accumulating that the H_4 receptor, to a much greater extent than the H_1 receptor, is implicated in some pruritic responses. Experiments employing models of allergic contact dermatitis have shown that although H_4 receptor antagonism fails to reduce the allergic inflammatory response, allergen-induced itch is strongly inhibited, and this suggests that a combination of H_4 and H_1 receptor antagonism might offer a valuable new approach for the treatment of pruritus-related conditions such as atopic dermatitis.

As work on the H_4 receptor progresses, evidence is accumulating for an immunomodulating role for histamine in the immune response, and there is much optimism that further insights into the role of H_4 receptors in immune and inflammatory disorders will yield novel therapeutic agents for the alleviation of a range of allergic conditions. Other demonstrations of the expression of functional histamine receptors on human T and dendritic cells also point to an immunomodulatory effect of histamine.

8.4.2 Early Studies on Opioid Drug-Induced Release of Histamine

In 1907 Windaus and Vogt completed the first chemical synthesis of histamine and soon after Sir Henry Dale and coworkers began investigations that showed that histamine was a powerful vasodepressant, it stimulated smooth muscle from the gut and respiratory tract and caused shock when injected into laboratory animals mimicking the systemic effects of anaphylaxis. These early results were followed by demonstration of the involvement of histamine in vascular reactions of the skin and the observation that morphine caused the so-called triple response in human skin, that is, the event sequence of an initial red spot followed by a red irregular flare and a fluid-filled wheal. Over 30 years later antihistamines were shown to reduce morphine-induced skin wheals, and histamine itself was detected in effluents of isolated perfused cat gastrocnemius muscle after arterial injection of opium alkaloids. Released histamine was also detected in cat skin, and raised levels were found in plasma after intravenous injection of morphine.

The significance of the release of histamine in humans stems from its actions of increasing both heart rate and the force of myocardial contraction, thus increasing cardiac output, and from its dilatator effect on small blood vessels that leads to flushing, decreased vascular resistance, and

Table 8.4 Monitoring cutaneous mast cell degranulation in vivo in humans. Histamine levels in venous blood following intradermal challenge with histamine, morphine, and antigen

Challenge agent	Time (min) to reach peak histamine level[a]	Range of histamine levels (ng/ml)	Time (min) to return to baseline[b]
Histamine (2 mg)	2–10	1.4–85.2	30
Morphine sulfate	1–8	2.3–12.7	30
Antigen	5–15	1.1–24.4	>60

Results summarized from McBride P et al. J Allergy Clin Immunol. 1989;83:374

[a]For all three challenges, histamine levels peaked 5–10 min before maximum development of wheal and flare reactions

[b]Histamine baseline levels, 0.6 ng/ml

hypotension. Despite the many early relevant demonstrations of the physiological actions of histamine in laboratory animals, and of morphine-induced release of the autacoid in human skin, an understanding of the clinical relevance of histamine liberation by opioid drugs was slow in coming. Studies in the late 1950s on urticaria pigmentosa linked opioids with the release of histamine and flushing of the skin and then in the early 1970s, studies in healthy humans demonstrated significant decreases in peripheral and total systemic vascular resistance following the injection of doses of up to 2 mg/kg of morphine.

8.4.3 Summary of Morphine-Induced Hemodynamic and Cutaneous Changes in Humans

Infusion studies designed to examine the relationship between human plasma histamine levels and symptoms revealed that from resting levels of 0.62 ng/ml, 1.61 ng/ml of histamine produced a 30 % increase in heart rate, 2.39 ng/ml elicited flush and headache, and 2.45 ng/ml was followed by a 30 % increase in pulse pressure. Venous blood plasma histamine levels were monitored after intradermal injection of morphine to induce cutaneous mast cell degranulation and the findings (Table 8.4) were compared with the results of intradermal challenge with histamine and antigen. Two interesting findings were that morphine liberated histamine concentrations sufficient to produce adverse symptoms and the time taken for histamine levels to return to baseline following antigen injection was over twice the 30-min period seen with histamine and morphine.

A review of the many human studies on the histamine releasing properties of OADs (the vast majority employing morphine) reveals not only significant progress in elucidating hemodynamic and cutaneous changes but also a picture that is sometimes confusing and contradictory and with some important questions still in need of convincing answers. Table 8.5 is an attempt to summarize results obtained from the most significant in vivo *and* in vitro studies designed to examine morphine-induced hemodynamic and cutaneous changes in patients over the last 35 years. Different findings of plasma histamine concentrations, peripheral and central hemodynamic vascular effects, and cutaneous changes can often be explained by the dose of opioid used, its mode and rate of administration, application of different methodologies for measuring the released histamine, the site of opioid injection, the distribution of histamine receptors, the effects of other medications, and the variation in patients' physiological responses to histamine. For a given dose of morphine, variations between individuals in plasma concentrations of histamine and hemodynamic and skin changes can be significant and extend over a wide range. Therefore, in reviewing the relevance of plasma histamine levels to hypotension, one needs to take into account that morphine itself has direct effects on the vascular, individual responses to histamine can vary widely, the relative magnitudes of histamine release and cardiovascular effects are unpredictable, and apparent correlations of morphine-induced histamine levels with decreases in systemic vascular resistance do not imply causation. Because of morphine's capacity to directly induce vascular responses, the use of antihistamines to antagonize the adverse hemodynamic effects of histamine-releasing opioids is not likely to be completely effective. As well as morphine's multiple direct effects on the

Table 8.5 Summary of studies on morphine-induced histamine release. Hemodynamic and skin test changes in patients and in vitro findings

Route of administration (or in vitro study) and dose (or concentration) of morphine used	Histamine release[a]	Hemodynamic and other effects[b]	Reference
Intravenous			
0.5 or 1 mg/kg rapid IV	NT[c]	↓ PVR[d], ↑ capacitance	Hsu et al. Anesthesiology. 1979;50:98
1 mg/kg IV at 5–10 mg/min	↑	↓ SVR, ↓ DBP, ↑ CI[e]	Philbin et al. Anesthesiology. 1981;55:292
0.3 mg/kg IV at 10 mg/min	↑	Hypotension, tachycardia, erythema ↓ SVR, ↑ CO, ↑ catecholamines	Fahmy. Anesthesiology. 1981;55:329
0.3 mg/kg IV at 5–10 mg/min	↑	↓ SVR, ↓ MAP, ↓ SBP, ↑ CO, ↑ HR, ↑ SV, ↑ plasma epinephrine	Fahmy et al. Clin Pharmacol Ther. 1983;33:615
1 mg/kg IV at 100 μg/kg/min over 10 min	↑[f]	↓ MAP, ↓ SVR[f]	Rosow et al. Anesthesiology. 1982;56:93
0.6 mg/kg IV over ≤ 10 min	↑[g]	Hypotension, tachycardia[g]	Flacke et al. Anesth Analg. 1987;66:723
1 mg/kg IV over 10 min	→	–	Warner et al. J Cardiothorac Vasc Anesth. 1991;5:481
0.16 mg/kg IV	↑[h]	–	Withington et al. Anesthesia. 1983;48:26
0.15 mg/kg IV	↑[i]	"Symptoms"[i] of histamine release but no hemodynamic changes	Doenicke et al. Clin Pharmacol Ther. 1995;58:81
0.07–0.14 mg/kg IV over 2 min	→[j]	↑ MAP[k], ↑ HR, → catecholamine	Mildh et al. Anesth Analg. 2000;91:51
Intradermal			
5×10^{-6} M to 1.5×10^{-3} M intradermal skin tests	↑[l]	wheal and flare	Levy et al. Anesthesiology. 1989;70:756
In vitro studies			
1.5×10^{-5} M to 4.5×10^{-3} M in vitro with leukocytes and skin mast cells	↑[m]	–	Hermens et al. Anesthesiology. 1985;62:124
10^{-5} M to 3×10^{-4} M in vitro with human basophils, skin, lung, and heart mast cells	↑[n]	–	Stellato et al. Anesthesiology. 1992;77:932 Marone et al. Ann Fr Anesth Reanim. 1993;12:116

Adapted from Baldo BA, Pham NH. Histamine-releasing and allergenic properties of opioid analgesic drugs: Resolving the two. Anaesth Intensive Care. 2012;40:216. With permission from Australian Society of Anaesthetists

[a]Histamine or effect elevated ↑; decreased ↓; unchanged →

[b]*CI* cardiac index, *CO* cardiac output, *DBP* diastolic blood pressure, *HR* heart rate, *MAP* mean arterial pressure, *PVR* peripheral vascular resistance, *SBP* systolic blood pressure, *SO* stroke volume, *SVR* systemic vascular resistance

[c]Not measured

[d]46 % increase at 2 min. Returned to control values at 9 min. When promethazine preceded morphine decrease in PVR was 25 %

[e]Minimal effects on responses by H_1 and H_2 blockers alone but significant attenuation by H_1+H_2

[f]Biggest decreases in SVR occurred in patients with highest levels of plasma histamine

[g]In 1 of 10 patients

[h]In 9 of 38 patients

[i]In 13 of 15 patients within 5 min of injection

[j]Local signs (redness, itching) at injection site

[k]Transient increase

[l]Relative to histamine control

[m]Histamine released from skin mast cells only—detected at 1.5×10^{-4} M and maximum at 5×10^{-4} M morphine

[n]From skin mast cells only

vasculature, other hemodynamically active and inflammation-producing mediators may be released along with histamine following morphine-induced mast cell degranulation and this too contributes to the variable response to it and some other OADs. In addition to histamine, other preformed biologically active mediators and proteins are liberated from mast cell granules. These include the proteases chymase and tryptase which cause tissue damage, serine esterases, cathepsin G, carboxypeptidase, eosinophil chemotactic factor, neutrophil chemotactic factor, some interleukins, the anticoagulant heparin, platelet-activating factor (PAF), and tumor necrosis factor, although most of the latter is newly synthesized by activated mast cells.

In addition to studying hemodynamic changes caused by liberated histamine, plasma epinephrine and norepinephrine levels have been investigated following injection of morphine. In a study of a patient who experienced an anaphylactoid response following the intravenous injection of morphine 0.3 mg/kg, plasma catecholamines were increased and this was accompanied by decreases in systemic vascular resistance and arterial blood pressure. In another study, intravenous morphine increased cardiac output, histamine, and epinephrine plasma concentrations and decreased arterial blood pressure and systemic vasculature resistance in adult subjects with no history of drug allergy or clinical evidence of cardiovascular, pulmonary, or metabolic disease.

Although most studies of opioid analgesics have been done with morphine, it is clear that not all opioid drugs show the same capacity to release histamine. In both in vivo studies in humans and in in vitro experiments with human leukocytes; basophils; and skin, lung, and heart mast cells, *fentanyl* caused no change in plasma histamine levels and nor did it, or oxymorphone, release the mediator from mast cells in vitro. The finding that naloxone did not release histamine from skin mast cells and did not inhibit morphine-induced histamine release suggested that the release of histamine by morphine is a direct degranulating effect that does not proceed via opioid receptor recognition. Overall, studies on fentanyl have revealed little or no release of histamine from mast cells and no release with associated cardiovascular effects. The consensus is that fentanyl, *sufentanil, alfentanil, and remifentanil* do not cause histamine release when normal doses of the drugs are given intravenously.

Apart from morphine and fentanyl, meperidine is the only other opioid analgesic that has been fairly well studied for histamine liberating properties. This synthetic opioid is a potent releaser of histamine. In the skin it induces itch and wheal and flare reactions and erythema, hypotension, tachycardia, and epinephrine release following intravenous injection. Of the other clinically important opioid analgesic drugs only oxycodone, oxymorphone, buprenorphine, alfentanil, remifentanil, sufentanil, methadone, and codeine have been studied to any extent. The histamine-releasing capacity of codeine is well known with the drug sometimes employed as a positive control alongside allergen extracts in skin tests.

Interestingly, some opioid drugs reveal some functional differences between human mast cells and basophils and between mast cells from different anatomical sites. This is illustrated by morphine's selective release of histamine and tryptase from human skin mast cells but not from lung, intestinal, and heart mast cells or from blood basophils, while *buprenorphine* releases histamine and tryptase from human lung but not skin mast cells. Despite the belief by some that high doses of injected morphine can induce an anaphylactoid reaction via the release of histamine from skin mast cells, the above observational differences may indicate why morphine rarely produces systemic responses resembling anaphylaxis even though it strongly induces histamine release in the skin and why opioid drug-induced direct histamine release does not, or rarely, produces bronchospasm. In fact, if bronchospasm does occur in an anaphylactoid reaction, the reaction is more likely to be immune-mediated (Sect. 7.3). Findings so far with mast cells derived from heart tissue indicate that the human heart may be both the site and target of anaphylactic reactions. Human heart mast cells are activated in vitro by a variety of drugs including some anesthetic agents and radiocontrast media to produce non-IgE-mediated "anaphylactic" reactions; anti-IgE,

anti-FcεI, and C5 induce the release of histamine and tryptase, and it has been suggested that anaphylactic reactions could be particularly severe in patients with certain cardiovascular diseases.

Because of its effects on the heart and vasculature, in particular its venodilation properties and its capacity to produce small reductions in heart rate, morphine is now often indicated in the initial treatment of acute coronary syndromes (ACS). Although no randomized, controlled trials have been undertaken, bodies such as the American Heart Association in a Scientific Statement of guidelines and under the heading "Management strategies: Basic therapy for acute coronary syndrome (ACS)" has recommended the administration of morphine when symptoms are not immediately relieved by nitroglycerine and a β-blocker or when acute pulmonary congestion or agitation is present. This relatively recent therapeutic application of morphine and its vasodilation effect in human veins also reminds us that the drug's direct effect on the peripheral arterial vasculature is not well understood. Recently, intra-arterial infusion of morphine was shown to elicit dose-dependent vasodilation mediated by histamine and nitric oxide. Thus, morphine can be considered to be a vasodilator of both arteries and veins reinforcing the belief that the opioid might be useful for the treatment of some cardiovascular diseases.

Unlike morphine, intravenous *tramadol* produces no change in plasma histamine concentration and no systemic anaphylactoid reactions or flushing of the skin. Only slight and transient elevations in blood pressure and heart rate without abnormal ECG findings have been noticed so the drug can be considered to be hemodynamically stable. Also, unlike nonsteroidal anti-inflammatory drugs, tramadol does not inhibit prostaglandin synthesis.

8.5 Allergenicity of Opioid Analgesic Drugs

Many of the symptoms elicited by histamine are the same or similar to those of antibody-mediated type I allergic responses and some other non-immediate hypersensitivity reactions (Sect. 3.2.5.1), and since many opioid drugs are potent releasers of histamine, confusion and difficulties arise in distinguishing and managing what presents as the same clinical picture, namely, anaphylactoid and anaphylactic reactions. When one considers the variable cutaneous histamine releasing properties of opioid analgesic from strong for morphine, codeine, and meperidine to weak or absent for fentanyl, difficulties involved in employing the drugs in skin tests at the diagnostic level are readily apparent. This has led to the recommendation that placebo-controlled challenge is necessary for the accurate diagnosis of allergic sensitivity to opioid drugs, but the drugs may sometimes be used for this purpose if diluted beyond their histamine releasing concentrations. Before considering the question of how opioid drug-induced anaphylactoid and anaphylactic reactions can be resolved, their allergenicity and frequency of allergic reactions will be considered.

One might assume that given the widespread prescribing and frequent administration as analgesics of morphine, codeine, their hydro- and oxy-derivatives, meperidine, fentanyl, and tramadol, and the use of heroin (often by intravenous injection) and methadone by addicts, IgE-antibody-mediated allergy to these drugs would not be uncommon. This is not the case. The reasons why are examined below.

8.5.1 Naturally Occurring and Semisynthetic Opioid Drugs

There is only a handful of reports of adverse reactions of the immediate kind following the administration, usually by intravenous injection, of morphine and other naturally occurring opioid drugs, but, because of the failure to apply basic diagnostic tests, a confident diagnosis distinguishing an anaphylactoid from an immune-mediated reaction is often not possible. In the most comprehensive and informative immunological study so far, IgE antibodies that reacted equally well with morphine and codeine were detected in the serum of a suspected anaphylactic

patient following intramuscular injection of papaveretum–hyoscine as preanesthetic medication. In the late 1980s when the reaction occurred, papaveretum, a mixture of opium alkaloids, was standardized to contain 47.5–52.5 % morphine, 2.5–5.0 % codeine, 16.0–22.0 % narcotine, and 2.5–7.0 % papaverine. Intradermal tests revealed positive wheal and flare responses to papaveretum 200 ng/ml and morphine 100 ng/ml (0.3×10^{-6} M) but negative results with hyoscine, meperidine, and fentanyl. Skin testing of normal subjects was not undertaken. Liberation of histamine by morphine from human skin mast cells in vitro and in human skin is first detected at 1.5×10^{-4} M and 5×10^{-6} M, respectively, so the skin test result with morphine probably reflected antibody-mediated histamine release by mast cells. Direct binding immunoassays employing morphine, codeine, and hyoscine solid phases to detect specific IgE antibodies revealed strong positive reactions to both opium alkaloids and a weak to equivocal reaction with hyoscine. Quantitative hapten inhibition studies with some analogs of morphine (Figs. 8.5 and 8.6) showed that the two best inhibitors, morphine and codeine, which differ only at position 3 where morphine has a hydroxyl and codeine a methoxy group, were of equal potency. This suggested that the IgE antibodies did not recognize this region of the morphine and codeine molecules. Some recognition of nalorphine was also obvious but the decrease in inhibitory potency indicated that the composition of the group attached to the piperidine nitrogen was also important for IgE antibody recognition. Evidence of the importance of the cyclohexenyl ring for antibody binding was the weak inhibition shown by naloxone that shares an *N*-propyl group with nalorphine but differs in having a keto group at C6, a single bond at C7–C8, and a hydroxyl group at C14. Further evidence of the importance of the substituent attached to the nitrogen was the lack of recognition of naltrexone which is structurally the same as naloxone except for the presence of an *N*-cyclopropylmethyl instead of an *N*-allyl substituent. Surprisingly, meperidine and methadone showed significant inhibition of IgE binding but like morphine and codeine, meperidine has an *N*-methyl and methadone a dimethylamino group. Analysis of the inhibition results suggested, therefore, that the important structural features of the morphine (and codeine) allergenic (that is, IgE antibody binding) determinant are the cyclohexenyl ring with a hydroxyl at C6 and, most importantly, a methyl substituent attached to the nitrogen (Fig. 8.7). These fine structural features of the morphine allergenic determinant are similar to the complementary structures recognized by antibodies to morphine raised in laboratory animals.

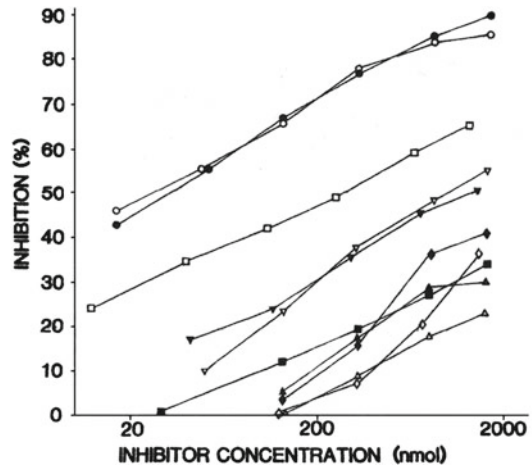
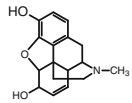
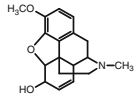
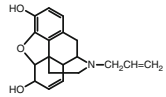
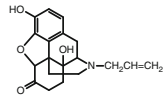
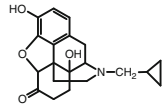
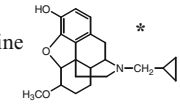
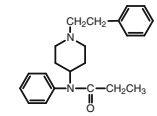
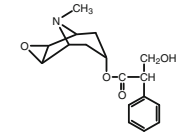
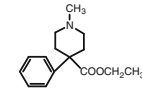
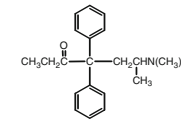


Fig. 8.5 Specific inhibition by morphine and some structurally related opioid drugs of IgE antibodies in the serum of a patient allergic to papaveretum. Drugs were used to inhibit antibody binding to a morphine–solid phase covalent complex. Symbols: (*open circle*) morphine, (*filled circle*) codeine, (*open square*) nalorphine, (*filled square*) naloxone, (*open triangle*) naltrexone, (*filled triangle*) buprenorphine, (*inverted open triangle*) meperidine, (*inverted filled triangle*) methadone, (*open diamond*) fentanyl, (*filled diamond*) hyoscine. From Harle DG et al. Anaphylaxis following Administration of Papaveretum. Case Report: Implication of IgE Antibodies that React with Morphine and Codeine, and Identification of an Allergenic Determinant. Anesthesiology. 1989;71:489. Reproduced with permission from Lippincott Williams & Wilkins

At normal doses, *codeine*, even more so than morphine and despite its capacity to release histamine in the skin (but perhaps also reflecting the differences in parenteral administration), has rarely been implicated in anaphylactoid or anaphylactic reactions. However, an unusual case of fever with urticaria, generalized pruritus, and

Compound	Structure	Inhibition (%) of IgE antibody binding to morphine-Sepharose with 200 nmol of compound
Morphine		72
Codeine		72
Nalorphine		46
Naloxone		15
Naltrexone		3
Buprenorphine	 *	31
Fentanyl		3
Hyoscine		9
Meperidine		31
Methadone		31

* *Buprenorphine contains an endoetheno bridge between C6 and C14 and a 1-hydroxy-1,2,2-trimethylpropyl substitution on C7.*

Fig. 8.6 Comparative inhibitory potencies of morphine and some other opioids in quantitative hapten inhibition studies with serum containing IgE antibodies to papaveretum (see Fig. 8.5). From Harle DG et al. Anaphylaxis following Administration of Papaveretum. Case Report: Implication of IgE Antibodies that React with Morphine and Codeine, and Identification of an Allergenic Determinant. Anesthesiology. 1989;71:489. Reproduced with permission from Lippincott Williams & Wilkins

erythema occurring 6 h after ingestion of a single tablet of 30 mg of codeine has been reported. Specificity of the reaction was confirmed by oral challenge 1 month later with 5 mg of codeine phosphate when the patient responded 3 h later with hot and cold sensations, oral pruritus, generalized urticaria, palpebral and labial edema, and petechia. At higher doses maculopapular and urticarial rashes, generalized dermatitis, and eczema are seen. Codeine has also been reported to cause pseudo scarlet fever and adverse reactions ranging from tachycardia and cutaneous vasodilation to severe hypotension and apnoea in children given codeine phosphate intravenously. *AMA Drug Evaluations*, the American Medical Association's evaluation of the most commonly prescribed drugs, concluded that codeine phosphate should not be used intravenously in children and, more recently, this recommendation has been extended to adults following the reporting of life-threatening hypotension in three patients given codeine phosphate intravenously.

Despite *heroin's* notoriety and its widespread misuse intravenously, allergic reactions to the drug appear to be far less common than one might expect. A diagnosis of anaphylaxis, confirmed by a positive tryptase test at the time and positive prick testing several weeks later, was made on a patient following intrathecal injection of diamorphine. In recent years there have been a number of reports of heroin inhalation inducing or exacerbating life-threatening asthma. In one case diagnosed by bronchial provocation testing, inhalation of heroin powder provoked allergy involving asthma and urticaria. No primary cause can be identified in a significant number of deaths

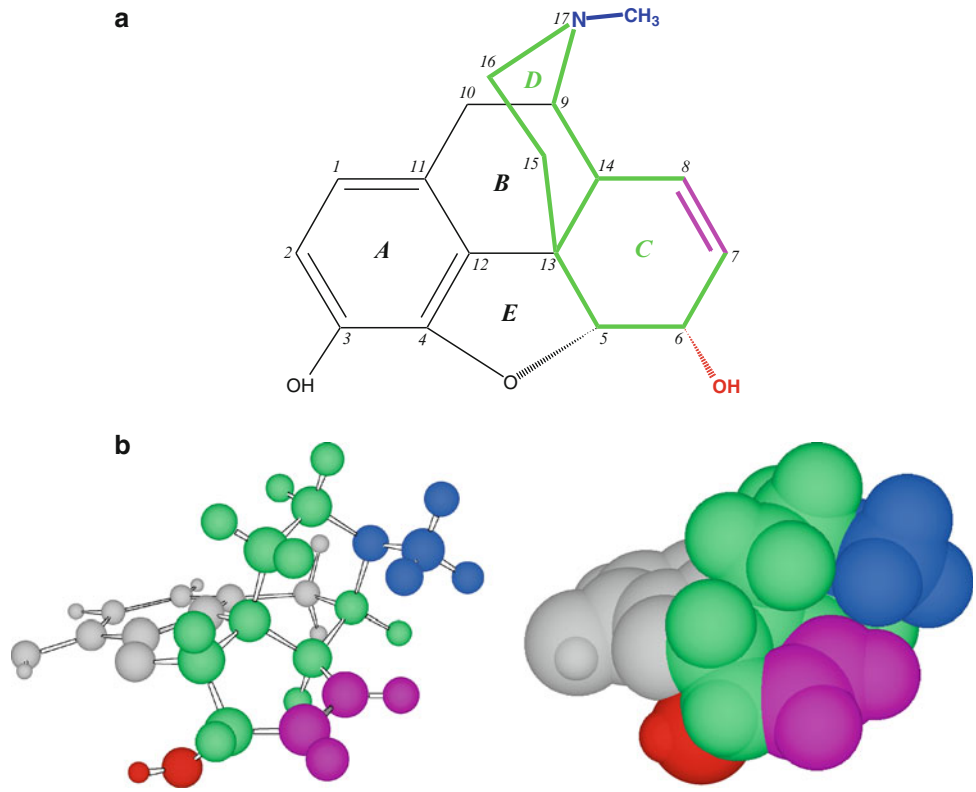


Fig. 8.7 Two-dimensional (**a**) and space-filling three-dimensional ball-and-stick (**b**, *left hand side*) and CPK (**b**, *right hand side*) molecular models of morphine showing in color the identified allergenic determinant recognized by the IgE antibodies in the papaveretum-allergic patient (refer Figs. 8.5 and 8.6). The individual fine-structural features of the determinant are shown in different colors: the piperidine ring (ring D) attached to the cyclohexenyl ring (ring C) are both shown in *green*; the double bond at positions 7–8 of ring C is in *magenta*; the hydroxyl at C6 is *red*; and the tertiary methylamine at position 17 is *blue*. From Baldo BA, Pham NH. Histamine-releasing and allergenic properties of opioid analgesic drugs: Resolving the two. Anaesth Intensive Care. 2012;40:216. Reproduced with permission from Australian Society of Anaesthetists

in heroin drug addicts and speculation that anaphylaxis might be involved in at least some cases remains. Morphine is known to bind to the gamma-globulin fraction of serum from addicts so it is speculated that its close analog diamorphine, complexed with serum proteins, might act as a sensitizing allergen in some users. Mast cell tryptase determinations on postmortem blood samples from addicts and in immunohistological investigations demonstrated elevated tryptase concentrations, and it was concluded that many of the fatalities might be due to drug-induced direct liberation of mediators from mast cells. However, given that tryptase release has not been totally disproved in some anaphylactoid reactions (refer Sects. 6.1.4.2 and 7.4.3.2), without tests for morphine- or heroin-specific IgE antibodies, there is still no direct evidence for the involvement of immunological processes, that is, type I allergy, in the deaths.

8.5.2 Synthetic Opioid Drugs

Meperidine, methadone, and fentanyl (and its close analogs alfentanil, remifentanil, and sufentanil) are widely used but again allergic reactions are rare. In what may be the first reported case of true type I allergy to *meperidine*, an intramuscular injection of 100 mg of the drug to a woman in

Table 8.6 Demonstration of cross-reactive recognition of morphine, meperidine, and fentanyl by human IgE antibodies[a]

	Serum 1		Serum 2	
Drug used for inhibition	Amount (nmol) for 50 % inhibition	Inhibition (%) of IgE-binding[b] with 200 nmol of drug	Amount (nmol) for 50 % inhibition	Inhibition (%) of IgE-binding[b] with 200 nmol of drug
Morphine	24	72	<1	98
Meperidine	830	31	22	74
Fentanyl	>2,000	3	43	62
Nalorphine	240	46	58	57
Naltrexone	>2,000	3	~800	17

Data adapted from Baldo BA et al. Chemistry of drug allergenicity. Curr Opin Allergy Clin Immunol. 2001;1:327. With permission from Wolters Kluwer Health

[a]IgE antibodies detected in two different sera from patients with suspected allergy to opioid analgesic drugs

[b]IgE antibodies detected by binding to a morphine–solid phase complex

labor induced a dramatic fall in blood pressure, cyanosis, and urticarial rash. A further 50 mg of meperidine after recovery again led to hypotension. Skin tests or specific IgE antibody assays have confirmed other immediate allergic reactions to meperidine, but in what appears to be the only claim of *methadone* allergy, no supporting diagnostic evidence was advanced to back up the belief that the observed localized urticaria was the result of a true type I allergy. Some IgE antibodies to morphine can also cross-react with meperidine and methadone (see previous section above and Figs. 8.5 and 8.6), while others react with meperidine and fentanyl. The latter finding was demonstrated in quantitative inhibition studies with two sera from subjects with "suspected narcotic allergy" (Table 8.6) and suggests that IgE antibodies that react with the synthetic opioid analgesics may occur more often than previously thought if suitable IgE direct binding and quantitative inhibition immunoassays are more widely employed to investigate suspected opioid allergic subjects. A serum from a subject examined for multiple drug allergies provided further evidence that methadone cross-reacts immunologically with morphine, and IgE antibodies can detect this. Antibodies in the subject's serum strongly recognized morphine, codeine, and methadone, reacted less strongly with nalorphine, and not at all with fentanyl and the antagonists naloxone and naltrexone (Fig. 8.8). The inhibitory potencies for morphine, codeine, and methadone were of the same order again indicating that, in rare cases, immunoglobulin E antibodies occur that detect cross-reactive allergenic determinants on the naturally occurring phenanthrene morphine and some of the opioid synthetic drugs.

Fentanyl, perhaps again reflecting usage, is the subject of most of the few reports of anaphylactoid/anaphylaxis reactions to synthetic opioid analgesic drugs, but in a number of cases again no appropriate diagnostic data were advanced to support the diagnosis of fentanyl-induced anaphylaxis. A curious feature noticed in two different anaphylactic reactions to fentanyl was an apparent delay between the administration of the drug and the appearance of symptoms. A 15-min gap occurred in one case while in the other, profound hypotension was observed only after an hour. In the latter case, although hypotension may have occurred immediately and not observed because it was successfully contained by the initial treatment, another possible explanation is the small amount of fentanyl-induced release as a result of the low concentration of the potent drug (compared to say morphine) acting at the mast cell membrane.

Tramadol is generally considered to be a "safe" drug with a low risk (put at less than 0.1 % in one report) of adverse reactions. Eleven cases of angioedema, possibly related to tramadol, were reported in Sweden and in 5 of 28 patients (18 %) with chronic urticaria exacerbated by nonsteroidal anti-inflammatory drugs, tramadol-induced urticaria with one of the patients developing laryngeal edema. Hypersensitivity

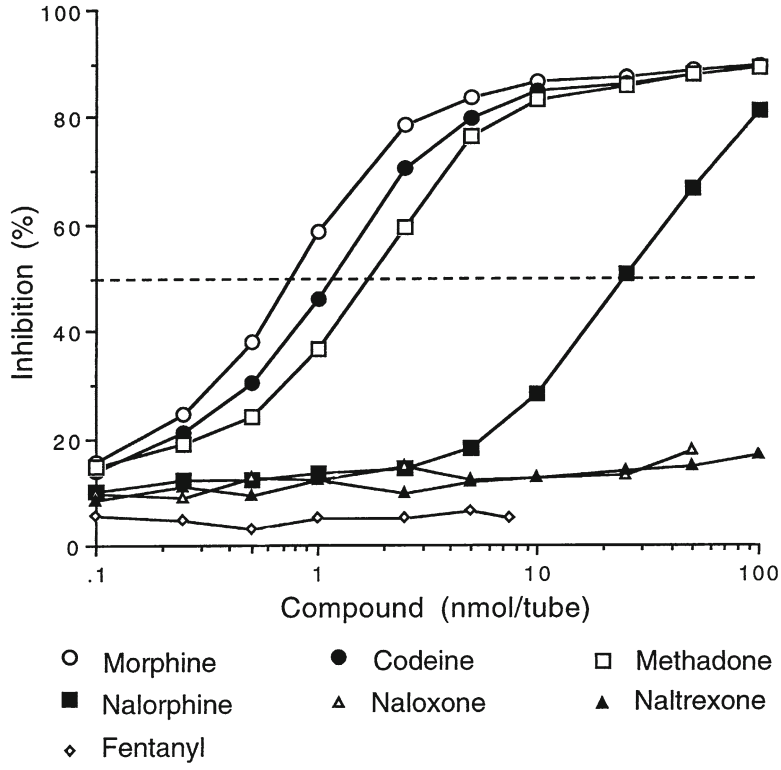


Fig. 8.8 Immunological cross-reactivity between methadone, morphine, and codeine demonstrated by serum IgE antibodies. Serum was from a patient showing multiple drug allergies. From Pham NH et al. Studies on the mechanism of multiple drug allergies. Structural basis of drug recognition. J Immunoassay Immunochem. 2001;22:47. Reproduced with permission from Taylor & Francis

pneumonitis and a maculopapulous toxic skin reaction with secondary erythroderma have also been ascribed to the drug. Assessment of the possible involvement of tramadol in Stevens–Johnson and Lyell's syndromes showed a high univariate relative risk for recent use, but comedication with highly suspected drugs led the authors of the study to "doubt causal association for tramadol." In general, skin reactions caused by tramadol are said to be uncommon and usually benign.

8.6 Resolving the Histamine-Releasing and Allergenic Effects in Diagnosing Reactions to Opioid Drugs

In reviewing the published findings on adverse reactions induced by all the opioid analgesics implicated so far, it is clear that the key to distinguishing a pseudoallergic reaction resulting from the direct release of histamine from a true anaphylactic reaction lies in the application of diagnostic investigations that reveal underlying immunological processes. Table 8.7 that summarizes a number of cases where reactions to opioid analgesics were described, compares the diagnostic conclusions of the authors of each study with a retrospective diagnostic assessment based on our necessary information requirements and tests for classifying a reaction as anaphylactic, that is, a true type I IgE antibody-mediated allergic response. These requirements (see also Chap. 4) are:

- A carefully gathered and recorded history and lists of the drugs administered and the drug-induced signs and symptoms.
- The use of appropriate dilutions of suspected drugs in skin tests (skin prick and/or intradermal tests) and/or placebo-controlled challenge

Table 8.7 Summary of some case studies of anaphylactoid/anaphylactic reactions to opioid analgesic drugs and diagnostic conclusions

Drug	Symptoms	Diagnostic tests employed	Test results	Authors' diagnosis	Retrospective assessment of diagnosis and comments
Morphine 0.3 mg/kg IV at 10 mg/min	Hypotension, tachycardia, flushing	IDT with morphine (0.35×10^{-2} M)	2 cm wheal	Anaphylactoid reaction. Direct effect of morphine. Type 1 reaction unlikely	Anaphylactoid reaction. No evidence of immune-mediated reaction
Morphine 10 mg bolus IV	↓ BP, ↑ HR, tachycardia	Tryptase test	Positive	Anaphylaxis	Possibly anaphylaxis but confirmatory tests lacking
Morphine 3 mg bolus IV	↓ BP, ↑ pulse, shortness of breath, wheezing	None	–	Anaphylactoid reaction	Anaphylactoid or anaphylactic. Absence of appropriate test results precludes confident diagnosis
Papaveretum–hyoseine IM	Hypotension, sweating, nausea, palpitations	IDT with morphine (0.3×10^{-6} M) IgE Abs with morphine–solid phase	1 cm wheal Morphine and codeine positive	Anaphylactic reaction	Anaphylactic reaction. Skin tests to check for irritant reaction not done
Fentanyl 50 μg	Delayed ↓ BP, erythema, urticarial rash, cyanosis	IDT with fentanyl 5 ng/ml 0.5 ng/ml	 5 mm wheal and flare 6 mm wheal and flare	Anaphylactic reaction	Anaphylactic reaction
Fentanyl 100 μg IV	↓ BP, sinus tachycardia, wheezing	Scratch and IDT	Positive wheal and flare to fentanyl and suxamethonium	Anaphylactic reaction to fentanyl	Anaphylactic but could be cross-reaction with MNBDs
Fentanyl (no details)	Breathing difficulties, wheezing, frothy secretions	LTT SPT	Positive for fentanyl and proposol Positive for meperidine	Anaphylaxis to fentanyl and propofol	Diagnosis not firmly established. Could be anaphylactoid. No positive skin test for fentanyl. Relevance of LTT and meperidine SPT? Conc. of meperidine SPT?
Meperidine 100 mg IM	↓ BP, ↓ pulse, cyanosis, urticarial rash	Challenge with 50 mg meperidine	↓ BP, face puffiness	"Hypersensitivity" to meperidine	Inconclusive. No immune basis identified to confidently diagnose anaphylaxis

↑ Elevated, ↓ decreased, *BP* blood pressure, *HR* heart rate, *IDT* intradermal test, *LTT* lymphocyte transformation test, *NMBD* neuromuscular blocking drug, *SPT* skin prick test

tests. Skin tests with OADs are carried out with the knowledge that they are, in general, histamine releasers; some are more potent in doing this than others and therefore the dilutions of drug used are critical. The concentrations (mg/ml and molarity) of the five most commonly used OADs that are normally nonreactive in the skin of normal, healthy subjects are for skin prick and intradermal testing, respectively:

Morphine 1 mg/ml, 3.5×10^{-3} M; 10 μg/ml, 3.5×10^{-5} to 10^{-7} M

Fentanyl 0.05 mg/ml, 1.5×10^{-4} M; 5 μg/ml, 1.5×10^{-5} M

Alfentanil 0.5 mg/ml, 1.2×10^{-3} M; 50 μg/ml, 1.2×10^{-4} M

Remifentanil 0.05 mg/ml, 1.3×10^{-4} M; 5 μg/ml, 1.3×10^{-5} M

Sufentanil 0.005 mg/ml, 1.3×10^{-5} M; 0.5 mg/ml, 1.3×10^{-6} M

A histamine control should be included and drugs should also be tested for histamine releasing/nonspecific irritant effects in the skin of normal subjects.

- Determination of serum tryptase levels sampled at appropriate time intervals preferably within 6–8 h of the reaction. More than one sample should be taken if possible.
- Application of specific IgE antibody immunoassays for individual drugs together with appropriate inhibition studies to confirm specificity and identify possible cross-reactive drugs.

The selected examples summarized in Table 8.7 are only a few of many case studies that show confusion with, or a misunderstanding of, the terms "anaphylactoid" and "anaphylactic." The studies reveal the often inadequate or even complete absence of, appropriate diagnostic investigations necessary for an accurate diagnosis. In the absence of evidence defining the underlining mechanism of a reaction, it is likely that some, if not many, reactions caused by direct release of histamine and those that were mediated by IgE antibodies have been misdiagnosed. This conclusion is supported by a search of the English and French language literatures over the 20-year period 1964–1984 and a retrospective assessment of 975 so-called "immediate anaphylactoid reactions" to parenterally administered anesthetic drugs where the mechanism was confirmed in only half of the patients.

8.7 Why Are Opioid Analgesic Drugs So Poorly Allergenic?

OADs are some of the most commonly administered drugs in hospitals and together with their frequent prescribing (e.g., morphine, codeine, and tramadol) in the community worldwide, and their rampant misuse by addicts, the question of why IgE-antibody-mediated allergic reactions to them are so rare is intriguing. Numerous surveys have shown that OADs account for only about 1 % of anaphylactic/anaphylactoid reactions during the perioperative period ranking them behind colloids, hypnotics, antibiotics, latex, and NMBDs, the most frequently involved group of agents. It has long been recognized that "small" organic molecules of molecular weight less than about 1,000 Da need to be presented to the antibody-forming cellular machinery as haptens bound to macromolecular carriers (usually proteins) for antibody formation to result, although this may not always hold true (see Sect. 3.1). Morphine is known to bind to the γ-globulin fraction of human serum, morphine-reactive antibodies have been found in normal, unimmunized subjects, and morphine coupled to carrier proteins have readily induced IgG and IgM antibody responses in laboratory animals. All this suggests that morphine, and probably other opioid drugs, are not lacking immunogenicity. Although there is only a single report of the identification of, and specificity study on, an opioid drug-reactive IgE antibody in a patient allergic to an OAD(s) (see Sect. 8.5.1 and Figs. 8.5, 8.6, and 8.7), this may be as much a reflection of the lack of immunochemically based investigations in the drug allergy field as an indication of the rarity of such antibodies. For allergen-induced release of the mediators of allergy from mast cells and basophils, cross-linking by multivalent allergens of FcεRI receptor-bound complementary IgE antibodies on the cell surfaces is required. Drugs

might do this if they are at least bivalent in terms of their allergenic determinant sites, for example the NMBDs, or if they are in a multivalent form bound to a macromolecular carrier (see Sects. 3.1.2 and 7.4.2.3). It seems likely, if not clear, that OADs are not allergenically bivalent and cross-linking of cell-bound IgE antibodies by any OAD has yet to be shown to occur.

Opioids are said to modulate both innate and acquired immunity, and among a vast array of claimed effects on immune cells, the drugs have been shown to suppress antibody formation by human B lymphocytes in vitro. Developments in this area of research may be relevant to the rare and infrequent occurrence of allergic antibodies to OADs in humans and are awaited with interest. A more conventional explanation than suppression of antibody formation for this apparent restricted immune response may be that OADs are seen as "self" components and are not seen as foreign antigens. Opioid peptides and the natural and synthetic OADs show the same biological activities in vivo and act on the same receptors, so it seems possible that from the immunological perspective, the shape and conformation in space of the endogenous and exogenous opioids might be seen as essentially the same.

8.8 Some Important Clinical Implications Related to the Use of Opioid Analgesic Drugs

Because the signs and symptoms of an OAD-induced pseudoallergic reaction and a true, antibody-mediated allergic reaction to an OAD can be so similar, distinguishing them is not always easy unless appropriate tests to identify an underlying immunological mechanism are applied. Routine application of skin tests with controls and/or placebo-controlled challenge tests with the suspected drug(s), serum tryptase determinations, and specific immunoassays to detect drug-specific IgE antibodies will take much of the uncertainty out of resolving the different responses.

If bronchospasm occurs in a reaction to an OAD, the reaction is more likely to involve OAD-reactive IgE antibodies. Likewise, cardiovascular collapse is more common in true anaphylactic responses and cutaneous symptoms more frequently seen in anaphylactoid reactions.

Historically, in prescribing an alternative OADs in cases of known existing hypersensitivity to an OAD, the phenanthrene drugs on the one hand and the synthetics meperidine, methadone, and fentanyl on the other have been grouped together, and it is usually recommended that a member of the opposite group should replace a known non-tolerated opioid. However, possible allergenic cross-reactivities among all of the OADs should be kept in mind. Although cross-reactivity between the structurally similar naturally occurring alkaloids morphine and codeine and their semisynthetic oxy- and hydro-derivative analogs is to be expected, cross-reactions between these morphine analogs and the synthetics meperidine, methadone, and fentanyl might be much more common than currently believed. Some compelling evidence for this has been presented (see above under Sect. 8.5.2). Before an alternative opioid drug is selected, it should be used to skin test the patient.

If appropriate OAD-reactive IgE antibody tests are available, they should be used as an adjunct to skin testing but an understanding of the immunochemical basis of cross-reactivity between opioids and NMBDs shows that a cautionary approach to interpretation is necessary. For example, a morphine solid phase is routinely employed to detect NMBD-reactive IgE antibodies in the sera of subjects allergic to NMBDs (see Sect. 7.4.3.4.2). The structural basis of the cross-recognition is the tertiary methyl ammonium group of morphine and the substituted ammonium groups on NMBDs.

When an OAD-induced reaction is known or suspected to be due to histamine release and the continued administration of an OAD is judged to be necessary, pretreatment with H_1 and H_2 (and, in future, perhaps an H_4) histamine antagonists is an option. Some opioid analgesics show differences in the amount of histamine they release and in the anatomical site where release occurs. Substitution of OADs can be made in both cases: for example, fentanyl for morphine in the former case and fentanyl or morphine for buprenorphine if histamine

release occurs in the lungs and fentanyl for morphine following cutaneous release. If an alternative to an OAD is sought, a nonsteroidal anti-inflammatory drug or regional analgesic techniques might be substituted.

Summary

Histamine releasing (anaphylactoid) and allergic (including anaphylactic) reactions to opioid analgesic drugs

- Anaphylactoid and anaphylactic reactions to OADs: same clinical picture; same immediate management
- Anaphylactoid and anaphylactic reactions are distinguished by the underlying mechanism: Anaphylactic reactions are immune (IgE antibody)-mediated; anaphylactoid reactions are not
- Reactions can be distinguished by appropriately diluted skin tests with suspected drug(s) and specific IgE antibody tests
- Tryptase assays are useful but may not distinguish anaphylactic and anaphylactoid reactions
- Potency of the direct histamine releasing effect of different OADs varies widely
- Direct release of histamine may provoke an anaphylactoid reaction especially if the OAD is given IV as a bolus, but correlation between plasma histamine levels and hypotension is poor
- OADs are poorly allergenic and anaphylactic and other less severe type I reactions are also rare
- Clinical manifestations are generally more severe and more often life-threatening in anaphylactic patients
- Cardiovascular collapse and bronchospasm are more frequent in anaphylactic reactions to OADs
- Cutaneous symptoms occur more often in anaphylactoid reactions
- OAD-induced histamine release rarely if ever produces bronchospasm
- Adverse skin reactions to OADs sometimes occur but as is the case for type I OAD allergic reactions, other hypersensitivity reactions are rare
- Tramadol is generally considered to be a 'safe' drug with a low risk (< 0.1%) of adverse reactions

Further Reading

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Nonsteroidal Anti-Inflammatory Drugs

9

Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are responsible for 20–25 % of ADRs. Cyclooxygenase isoenzymes COX-1 and COX-2 catalyze the formation of PGG_2 from arachidonic acid. Ultimate products of the metabolic pathway are PGD_2, PGE_2, $PGF_2\alpha$, PGI_2, and TXA_2. NSAIDs can be classified on the basis of their COX inhibitory and selective properties. Most are mainly COX-1 inhibitory, e.g., aspirin and ibuprofen, while some are COX-2 inhibitory, e.g., celecoxib. NSAIDs with the highest GI toxicity have the highest COX-1 selectivity. COX-2 is expressed in inflammation and selective COX-2 inhibitors show fewer GI effects but can produce cardiovascular effects. The mechanism of NSAID-induced respiratory reactions appears to be due to the redirection of arachidonic acid metabolism from the COX to the lipoxygenase synthetic pathway with associated production of cysteinyl leukotrienes. Aspirin-induced asthma, which makes up 3–5 % of adult asthmatics, has symptoms of chronic asthma, rhinosinusitis, and nasal polyps. NSAID-induced cutaneous reactions occur in a number of different clinical patterns. Challenge testing is the only way to diagnose sensitivity to an NSAID. Desensitization can be induced by repeated oral administration. Delayed reactions are seen and may take the form of contact dermatitis, FDE, DRESS, AGEP, SJS, TEN, or nephritis.

Anti-inflammatory drugs have a special, if not pivotal, place in the history of Western medicine and drug therapy and in the birth and evolution of the pharmaceutical industry. From at least the time of early Chinese and Egyptian civilizations over 5,000 years ago, humans sought plants and other natural materials for the relief of the four signs of inflammation—redness, heat, swelling, and pain. We know that as early as 400 BC, the Greek physician Hippocrates advocated the use of willow bark and leaves as an analgesic. During the time of Galen and thereafter, willow became widely used throughout the Roman world, an important component of the materia medica of Western medicine and a standard entry in the early pharmacopoeias. After more than two millennia of the use of barks of various *Salix* spp. for pain, fever, and inflammation in both Europe and the new world, salicin was isolated in 1828 and by the mid to late nineteenth century the

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_9, © Springer Science+Business Media, LLC 2013

properties and value of salicylate medicine were becoming increasingly appreciated. The chemical and early pharmacological work on salicin, salicylic acid, and sodium salicylate led to the production of salicylic acid by 1874 and the introduction of acetylsalicylic acid, named aspirin, by Bayer in 1899. In the twentieth century, other organic acids and later nonacidic anti-inflammatory compounds followed and, although the mechanisms of action of aspirin and other anti-inflammatories in the pre-prostaglandin age remained obscure, usage of what became known as the nonsteroidal anti-inflammatory drugs (NSAIDs) grew until this group was among the most commonly used drugs worldwide.

Severe bronchospasm following the ingestion of aspirin was described soon after its introduction. Now, with NSAIDs said to be used by more than 30 million people every day, 111 million prescriptions written annually in the USA and the drugs making up approximately 60 % of the USA over-the-counter analgesic market, NSAIDs are said to be responsible for 21–25 % of reported adverse drug events. This chapter will present a summary of important NSAIDs, their mechanism of action, the clinical manifestations of NSAID sensitivity reactions, diagnostic tools for, and management of, the reactions, and the pathomechanisms thought to be involved. The therapeutic and chemical properties of these drugs and their mechanism of action are presented before the clinical picture and pathogenesis of intolerances to NSAIDs are discussed later in this chapter.

9.1 Therapeutic Applications of NSAIDs

NSAIDs have analgesic and anti-inflammatory properties and are, therefore, widely used for the treatment of both minor and more severe pain and for the management of swelling and joint inflammation. Some NSAIDs also possess antipyretic activity and are, therefore, useful for the treatment of fever. Specifically, NSAIDs available over the counter such as aspirin and ibuprofen are heavily used for self-medication of a host of conditions involving pain including headache, migraine, pain due to inflammation and tissue injury, dysmenorrhoea, muscle strains, surgical, dental and postoperative pain, and so on. The drugs are also commonly taken for colds and influenza to relieve fever, temperature, and pain. Important indications for prescribed NSAIDs are rheumatoid arthritis and osteoarthritis where, in the former case, anti-inflammatory action is reflected in reductions of pain, swelling of joints and morning stiffness, increased mobility, and enhanced functional capacity (such as grip strength). No single NSAID has stood out as a superior drug for rheumatoid arthritis, rather, individual patients may respond better or worse to certain members of the group. For osteoarthritis, improvements may occur in pain at rest, stiffness, pain from movement, and night pain, and movement overall may become easier. Again, no individual NSAID seems superior, but indomethacin should be avoided because of its greater toxicity and its possible potential for accelerating progression of the disease. NSAIDs are also widely administered for inflammatory arthropathies such as ankylosing spondylitis and Reiter's syndrome, for acute gout, metastatic bone pain, renal colic, and postoperative pain, sometimes as a substitute for poorly tolerated opioid analgesics.

9.2 Patient Usage

Over-the-counter usage of NSAIDs is common in most countries, although one survey reported that about half of NSAID users were unaware of the drugs' potential side effects; over-dosing is more common with over-the-counter than with prescription users and approximately 40 % of prescription users take over-the-counter NSAIDs at the same time. A survey in the USA found that approximately 30 % of adults take over-the-counter analgesics on a regular basis for chronic pain. Use for this purpose increases with age—an estimated 10–40 % of people over 65 years take prescribed or over-the-counter NSAIDs on a daily basis. Ibuprofen and diclofenac are the most

commonly consumed NSAIDs throughout the world accounting for 40 % of global sales. Following, in order, are naproxen, meloxicam, celecoxib, ketoprofen, and etorocoxib. For prescription drugs, ibuprofen and naproxen head the list in the USA while diclofenac is the leader in the UK. Ibuprofen leads over-the-counter NSAID sales in the USA.

An extraordinary increase in NSAID use occurred with the introduction of the COX-2-selective drugs but, following the withdrawal of rofecoxib and valdecoxib in 2004–2005, there was a marked reduction in the prescribing of all NSAIDs. Between 2003 and 2005, the decrease in usage of COX-2-selective agents for arthritis in the USA went from 55.1 to 29.2 % while in Germany, COX-2 prescription numbers dropped by 37.1 million between 2004 and 2005, and overall NSAID usage decreased by 8.4 %.

9.3 Classification of NSAIDs

It is informative to classify NSAIDs on the bases of both their pharmacological and chemical properties but, before considering pharmacological mechanisms of these drugs, a classification based on chemical structures is presented. Many NSAIDs are organic acids with p*K*as between about three and five. Many are carboxylic acids and therefore form salts that ionize at physiological pH. The acidic group also accounts for the ionic binding with plasma proteins generally seen with NSAIDs, and it serves as a major site for the metabolism and clearance (e.g., as glucuronides) of the drugs. By virtue of their aryl groups and hydrophobic substituents, NSAIDs show varying degrees of lipophilicity.

9.3.1 Salicylates

The salicylate NSAIDs, for example, aspirin, diflunisal, and salsalate, are derived from the relatively strong organic acid, salicylic acid, used first as the sodium salt and then replaced at the turn of the nineteenth century by its acetylated derivative, acetylsalicylic acid or aspirin (Fig. 9.1a). Esterification of the phenolic hydroxyl group and substitution of a hydrophobic group at C-5 enhances the pharmacological action of the anti-inflammatory salicylates.

9.3.2 Propionic Acid Derivatives

These drugs are sometimes referred to as "profens," the term originating from the name of the group's original member, ibuprofen (Fig. 9.1b). Like the salicylates, they are organic acids (p*K*a = 3–5) and form soluble salts. Other members of the group include naproxen, fenoprofen, ketoprofen, dexketoprofen, carprofen, flurbiprofen, dexibuprofen, loxoprofen, and oxaprozin. Although the drugs, except for naproxen, are usually marketed as racemates, in vivo they invert from the inactive R-enantiomer to the active S-enantiomer and this is the isomer found in plasma.

9.3.3 Aryl and Heteroaryl Acetic Acids

This group can be further subdivided into indene/indoles, pyrroles, and oxazoles. Indomethacin and sundilac are the most well known of the indene/indole group. Indomethacin (Fig. 9.1c) is administered for rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis and to suppress uterine contraction but has a number of undesirable actions including gastrointestinal ulceration and hemorrhage, CNS effects, aplastic anemia, bone marrow suppression, and thrombocytopenia. Sulindac (Clinoril®), which lacks the indole nitrogen of indomethacin, is a prodrug that is 50 times as active in its metabolized sulfide form. A third well known drug in this group is etodolac (Lodine®). Tolmetin (Tolectin®), with a short half-life of less than 5 h is perhaps the best known pyrrole acetic acid NSAID while oxaprozin (Daypro®) is an example from the oxazole class of acetic acids.

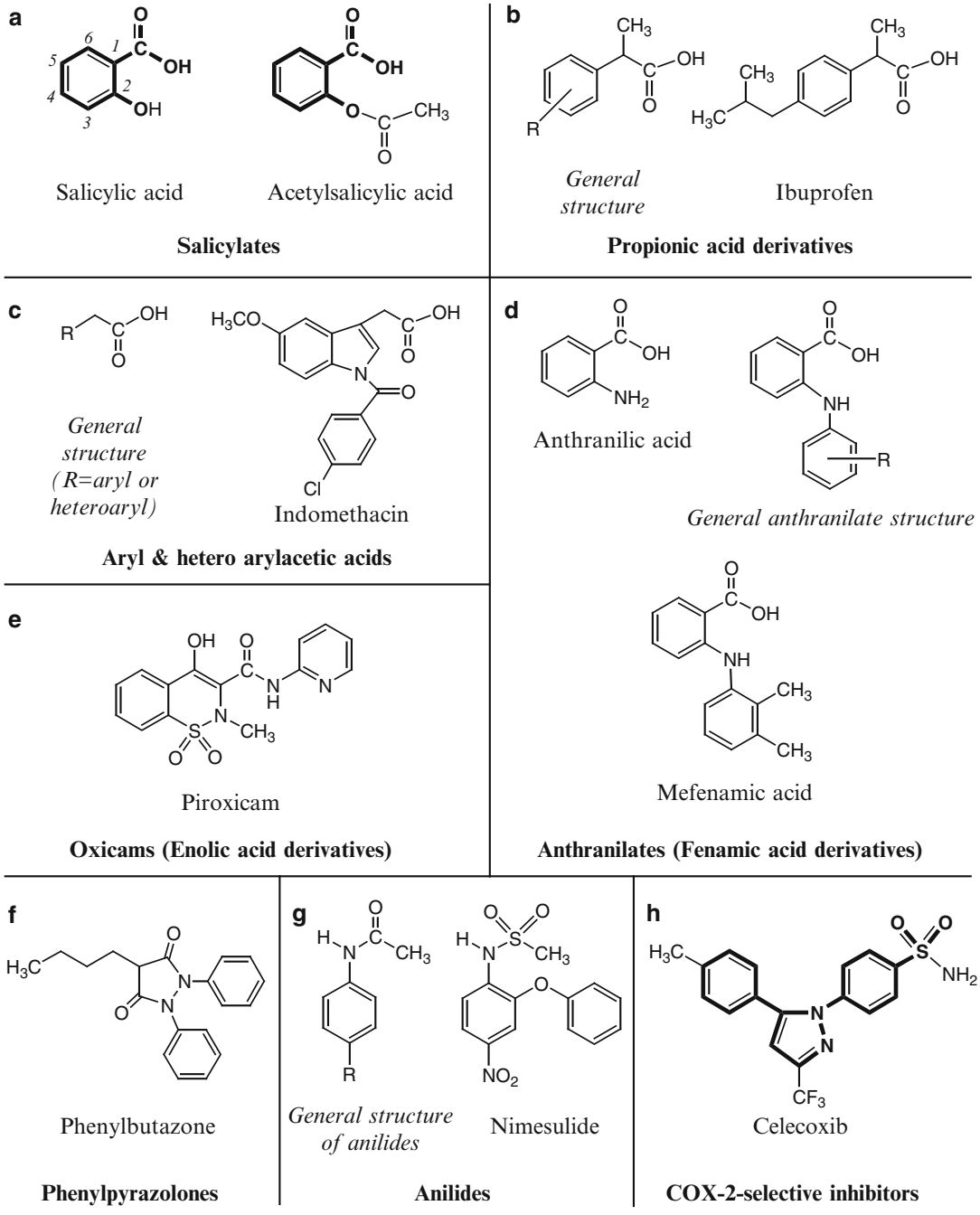

Fig. 9.1 Classification of nonsteroidal anti-inflammatory drugs based on chemical structure with important examples from each category. The structure common to salicylic acid and acetylsalicylic acid (aspirin) is *highlighted in bold* in category a and the central core structure of a diaryl-5-membered heterocyclic ring with two adjacent phenyl substituents is highlighted in category h

9.3.4 Anthranilates (Fenamic Acid Derivatives)

Also known as fenamates, these compounds, which include mefenamic acid (Ponstel®; Ponstan®) (Fig. 9.1d) and meclofenamic acid (Meclomen), are *N*-aryl derivatives of anthranilic acid. Note that anthranilic acid is a bioisostere of salicylic acid and diclofenac is a derivative of 2-arylacetic acid. The presence of small alkyl groups or halogen atoms on the *N*-aryl ring increases activity, for example, meclofenamate with Cl at positions 2, 5, and 6 on the ring is 25 times as potent as mefenamic acid. Other anthranilates administered are flufenamic acid and tolfenamic acid. The anthranilate NSAIDs are well absorbed from the gastrointestinal tract but produce a number of adverse reactions including ulceration, diarrhea, nausea, vomiting, headache, and hematopoietic toxicity.

9.3.5 Oxicams (Enolic Acid Derivatives)

The usual carboxyl group seen in many NSAIDs is absent from the oxicams which, for compounds such as piroxicam and meloxicam, derive their acidity from the 4-hydroxyl group. Both of these drugs are characterized by a 4-hydroxybenzothiazine group as shown in the structure of piroxicam (Fig. 9.1e). Lack of a carboxyl substituent means that the drugs cannot be readily glucuronidated and excreted, and this results in a longer half-life and once a day dosing. Other administered drugs in this group are tenoxicam, droxicam, lornoxicam, and isoxicam.

9.3.6 Phenylpyrazolones

Viewing aspirin as the progenitor drug, the first member of what we now call the NSAIDs is phenylbutazone (Fig. 9.1f), a compound with a 1-aryl-3,5-pyrazolidinedione structure. Introduced by Geigy in Basel in 1946, phenylbutazone was extensively used for over 30 years for arthritis and other pain until replaced by newer NSAIDs and because of its adverse effects that included agranulocytosis, bone marrow suppression, and bleeding. Oxyphenbutazone, a metabolite of phenylbutazone was also used as a NSAID. Other phenylpyrazolones in use include phenazone, propyphenazone, aminophenazone, and metamizole.

9.3.7 Anilides

These drugs are simple acetamides of aniline (Fig. 9.1g) with the heavily used acetaminophen (paracetamol) and phenacetin (now rarely, if ever, used) being the best known examples. They are neutral compounds without an acid group and with little if any inhibitory activity for cyclooxygenase. Their mechanism of action is different to other NSAIDs and their classification here along with other groups of NSAIDs is not a perfect fit. Nevertheless, acetaminophen is now an important and widely used drug and is often substituted for NSAIDs that are not tolerated (see below). Discovered as part of a program to identify anti-inflammatory drugs based on sulfonamides and developed as a clinically effective drug in the 1960s before the COX-isoforms were discovered, nimesulide (4-nitro-2-phenoxymethane sulfonanilide) (Fig. 9.1g) is a COX-2-selective NSAID with analgesic and antipyretic properties used for a number of conditions including the treatment of acute pain and osteoarthritis. This drug, now in the European Pharmacopoeia, appears to have more than one mechanism of action in addition to its inhibitory activity of the COX-2 isoenzyme. In a 2006 assessment of the clinical and safety profiles of numesulide, it was concluded that the drug is an effective and safe therapeutic choice for the treatment of various painful inflammatory conditions, and it has a rapid onset of action and an overall positive to risk profile.

9.3.8 COX-2 Selective Inhibitors

The enzymes that catalyze the synthesis of prostaglandins from arachidonic acid are known as cyclooxygenases, and the term COX refers to cyclooxygenase activity. By convention, the enzyme responsible for the production of

prostaglandins and thromboxane A_2, important for the regulation of stomach and kidney blood flow and gastric acid secretion as well as homeostatic maintenance, is termed COX-1. COX-2, induced by various inflammatory stimuli, is also responsible for prostaglandin production and plays an important part in pain and inflammatory processes. COX-2 inhibitors (often termed coxibs from *cox*-inh*ib*itors) are diaryl-substituted pyrazoles/furanones/oxazoles. Celecoxib (Fig. 9.1h), for example, has a central pyrazole ring connected to two phenyl rings, one with a methyl and the other a sulfonamide group. Rofecoxib has a central furanone ring and valdecoxib a central oxazole ring. The diaryl tricyclic structure with a five-membered heterocyclic ring and a phenyl ring with a sulfonamide or methylsulfone substituent are critical in determining specific COX-2 inhibition. The central core of a diaryl-5-membered heterocyclic ring with two attached phenyl rings found in the COX-2 selective inhibitors is highlighted in Fig. 9.1h. Coxibs have high pKa values (8–9) compared to conventional NSAIDs, are well absorbed providing peak plasma levels within 3 h, and have a relatively long duration of action due to slow clearance. COX-2 inhibitors are used to treat pain and inflammation in acute and chronic conditions like rheumatoid arthritis, osteoarthritis, surgical, dental and postoperative pain, acute injuries, and dysmenorrhea. Besides celecoxib, other COX-2 inhibitors include rofecoxib, valdecoxib, parecoxib, etoricoxib, firocoxib, and lumiracoxib. Due to the induction of a so-called cardio-renal syndrome involving an apparent high risk of myocardial infarction, exacerbation of hypertension and elevation of blood pressure, rofecoxib, and valdecoxib were withdrawn from the market a relatively short time after their introduction.

9.4 Mechanism of Action of NSAIDs

Prostanoids, the prostaglandins, and thromboxane are end products of fatty acid metabolism with important physiological functions and pathological effects in areas relevant to pain, inflammation, pyrexia, asthma, osteoporosis, cardiovascular disease, uterine function, and cancer. NSAIDs, a chemically diverse range of drugs with common analgesic, anti-inflammatory, and antipyretic properties exert their pharmacological action by interfering in the arachidonic biosynthetic pathways that produce the prostanoids.

9.4.1 Biosynthesis of the Prostanoids

Arachidonic acid, an unsaturated 20-carbon fatty acid located in the cell membrane as a phospholipid is released by either secretory or cytoplasmic phospholipases ($sPLA_2$ or $cPLA_2$). Cyclooxygenase (COX) or prostaglandin H_2 synthase (PGHS) catalyses the first two steps in the biosynthesis. There are two PGHS isoenzymes, PGHS-1 (COX-1; prostaglandin H synthase 1) and PGHS-2 (COX-2; prostaglandin H synthase 2) which exhibit two different but complementary enzymatic activities—a cyclooxygenase that catalyzes the formation of the hydroperoxy endoperoxide prostaglandin G_2 (PGG_2) from arachidonic acid and two molecules of oxygen (O_2) and a peroxidase that facilitates its subsequent reduction to the hydroxyendoperoxide PGH_2. PGH_2 is then converted by specific synthases and isomerases to five biologically active primary prostanoids, PGD_2, PGE_2, $PGF_2\alpha$, prostacyclin PGI_2, and thromboxane A_2, TXA_2 (Fig. 9.2). Whereas TXA_2 is a vasoconstrictor and stimulates platelet activation and aggregation, PGI_2 is a vasodilator that inhibits platelet activation. This suggests that in relation to the vascular system, the two highly biologically active molecules participate in a balancing process of homeostasis that normally protects against vascular damage.

9.4.2 The Cyclooxygenase Isoforms COX-1 and COX-2

In 1971, Vane and Piper found that NSAIDs inhibit the biosynthesis of prostaglandins from arachidonic acid by preventing the substrate from binding to the COX enzyme active site. As it was

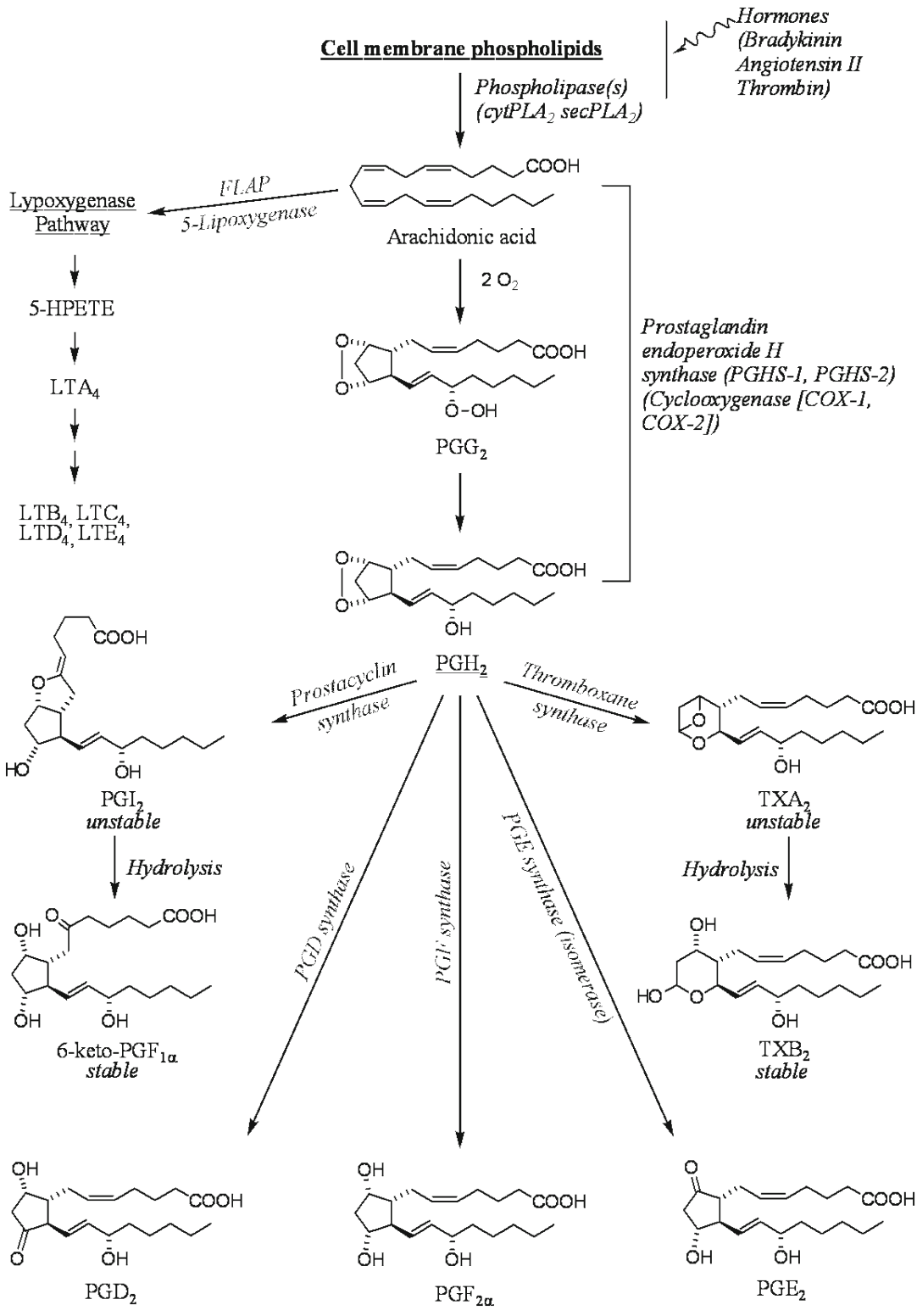

Fig. 9.2 Biosynthetic pathways of the synthesis of prostanoids from arachidonic acid by isoenzymes COX-1 and COX-2 catalysis

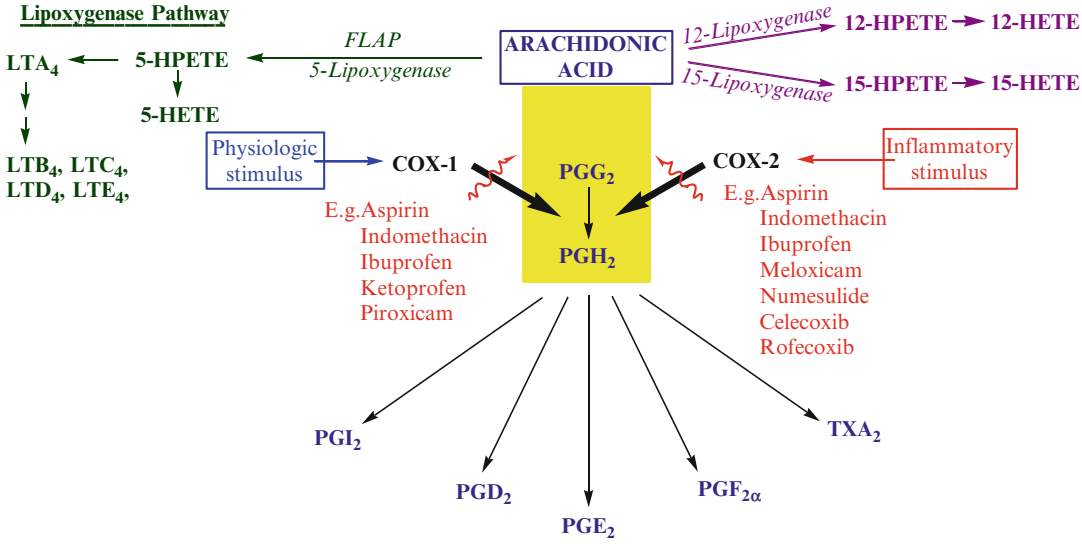

Fig. 9.3 Overall scheme summarizing the relationship between arachidonic acid and the cyclooxygenase, 5-lipoxygenase, and 15- (and 12-) lipoxygenase (eoxin) biosynthetic pathways together with intervention of COX inhibitors and their selectivity properties

known then, the COX enzyme was purified in 1976 before being cloned in 1988. Soon after in the early 1990s a second isoform of COX, now termed COX-2, was discovered and, as Vane has pointed out, in a clear sign of the therapeutic potential of this work, several highly effective anti-inflammatory agents and new therapeutic areas became the subject of investigation within 5 years of the discovery. COX-1 is constitutively expressed at high levels in the endothelium, platelets, monocytes, renal tubules, and seminal vesicles indicating that the prostanoids it helps to produce are involved in numerous physiological functions in the gastrointestinal tract, kidney, and platelets. For example, inhibition of COX-1 reduces the production of protective PGE_2 and PGI_2 in the stomach, which may lead to gastric ulceration. COX-2 expression is induced by cytokines and other inflammatory mediators such as interleukin (IL)-1, tumor necrosis factor (TNF), and lipopolysaccharides (LPS) in tissues and cells as diverse as macrophages, monocytes, endothelial cells, vascular endothelial cells and rheumatoid synovial endothelial cells. Since COX-2 is expressed only in inflammation, selective COX-2 inhibitors might be expected to show fewer side effects, for example, less gastric ulceration, but these drugs can produce cardiovascular effects. This is thought to be due to suppression of the cardio-protective PGI_2. In addition, and unlike the consequences of inhibiting of COX-1, PGE_2 and PGI_2 produced via the COX-2 pathway add to the inflammation initiated by mediators such as histamine and bradykinin leading to increased vascular permeability and edema.

9.4.3 Classification of NSAIDs by COX Isoenzyme Selectivity

NSAIDs can be classified on the basis of their COX inhibitory and selectivity properties. In general, most NSAIDs inhibit both COX-1 and COX-2 to some extent, but most are mainly COX-1-selective (e.g., aspirin, sulindac, indomethacin, ketoprofen, piroxicam); some are somewhat selective for COX-1 (e.g., ibuprofen, diclofenac, naproxen); others are slightly selective for COX-2 (etodolac, meloxicam); while NSAIDs like lumiracoxib, rofecoxib, and celecoxib are clear selective inhibitors of COX-2 (Fig. 9.3). Clearly, knowing the relative inhibitory potencies of different NSAIDs for COX-1 and COX-2 would be useful in attempting to

Table 9.1 Classification of NSAIDs according to their inhibitory potencies of COX-1 and COX-2[a]

Group	Inhibitory potencies of NSAIDs for COX-1 and COX-2[b]	Examples of NSAID
1	Full inhibition of COX-1 and COX-2 with poor selectivity	Aspirin, ibuprofen, diclofenac, naproxen, indomethacin, sulindac, piroxicam[c]
2	Inhibition of COX-1 and COX-2 but preferential selectivity for COX-2	Etodolac, meloxicam, nimesulide, celecoxib
3	Weak activity for COX-1; strong inhibition of COX-2	Rofecoxib, [diisopropylfluorophosphate]
4	Weak inhibitors of COX-1 and COX-2	Many salicylates, nabumetone, sulfasalazine

Results taken from Warner TD et al. Proc Natl Acad Sci. 1999;96:7563
[a]Human whole blood assays measuring formation of TXB_2 and PGE_2 by radioimmunoassay
[b]Calculated as IC_{50} and IC_{80} values (refer Table 9.2)
[c]Group 1 contains most of the currently used NSAIDs

establish COX-1 and COX-2 selectivities and distinguishing beneficial from harmful effects of the different drugs. This comparison was undertaken by the Vane group using human whole blood assays for COX-1 (with added NSAID and calcium ionophore A23187) and COX-2 (treated with aspirin to inactivate COX-1 and then LPS plus NSAID or exposed to IL-1β plus NSAID, then A23187 and diclofenac). Concentrations of TXB_2 (as a measure of TXA_2 formation) and PGE_2 were determined by radioimmunoassay as measures of COX activity. The NSAIDs tested could be divided into four groups on the basis of their inhibitory potencies of COX-1 and COX-2 (Table 9.1). Determination of the concentrations of individual NSAIDs needed to inhibit the COX-1 and COX-2 isoforms and comparison of the activities on a molar basis revealed the COX-1 and COX-2 selectivities of each drug and enabled their ranking in order of potency (Table 9.2). A comparison of these findings with epidemiological studies of NSAID-induced gastrointestinal toxicity revealed that NSAIDs associated with the highest gastrointestinal toxicity have the greatest COX-1 selectivity. Surveys have shown this order of toxicity (from the least to the most damaging NSAID) to be: ibuprofen, diclofenac, diflunisil, fenoprofen, aspirin, sulindac, naproxen, indomethacin, piroxicam, ketoprofen, tolmetin. NSAIDs associated with the greatest COX-1 selectivity in group 1 in the table (Table 9.2), viz., ketorolac, flurbiprofen, ketoprofen, and indomethacin, are among the NSAIDs with the greatest gastrointestinal toxicity. Ketorolac, the most COX-1-selective drug in the study, is known to be five times more toxic in this respect than any other NSAID. Group 2 contains preferential COX-2 inhibitors with, for example, etodolac and meloxicam producing 80 % inhibition of COX-2 and only 25 % inhibition of COX-1 at the same concentration. Compounds in this group have an improved gastrointestinal toxicity profile but, since they do inhibit COX-1 to some extent, increases in dosage could increase gastrointestinal toxicity. NSAIDs in group 3 have very little effect on COX-1 and this is reflected in their low gastrointestinal toxicity, but attention has been drawn to the possibility that their use in the presence of existing gastrointestinal damage may slow the healing process due to inhibited production of protective COX-2 products. Compounds in group 4 showed weak activity in inhibiting prostanoid production—sodium salicylate, for example, inhibited prostanoid formation at far higher concentrations than those achieved in vivo.

9.4.4 The So-Called COX-3 Isoform

After discovery of a COX-1 variant in some regions of the brain, it was suggested that acetaminophen, a weak inhibitor of COX-1 and COX-2, acts by inhibiting this variant, termed COX-3. Discovered in 2002, encoded by the COX-1 gene and with an intron not retained in COX-1, COX-3 was shown to be inhibited by acetaminophen, phenacetin, metamizole (dipyrone), antipyrine, and some NSAIDs in rodent experiments. It turned out, however, that results with canine COX-3 were not applicable to mouse

Table 9.2 COX selectivities of some important NSAIDs determined from their inhibitory potencies of COX-1 and COX-2

	Whole blood assay[a] IC_{80} (μM)[b]		COX selectivity[c] index IC_{80} ratio	Rank order[d] of selectivity for	
NSAID	COX-1	COX-2	COX-2:COX-1	COX-2	COX-1
GROUP 1[e]					
Aspirin	8.0	30.0	3.8	16	5
Diclofenac	1.0	0.23	0.23	7	14
Fenopropen	230	24.0	1.0	12	9
Flubiprofen	1.0	51.0	51.0	19	2
Ibuprofen	58.0	150.0	2.6	14	7
Indomethacin	0.46	2.0	4.3	17	4
Ketoprofen	1.0	6.0	6.0	18	3
Ketorolac	0.0034	1.0	294.0	20	1
Naproxen	110.0	330.0	3.0	15	6
Piroxicam	15.0	7.0	0.47	9	12
Sulindac sulfide	38.0	11.0	0.29	8	13
GROUP 2[e]					
Celecoxib	28.0	3.0	0.11	5	16
Etodolac	69.0	3.0	0.043	3	18
Meloxicam	22.0	2.0	0.091	4	17
Nimesulide	41.0	7.0	0.17	6	15
GROUP 3[e]					
NS398	65.0	1.0	0.015	1	20
Rofecoxib	>100	5.0	<0.05	2	19
GROUP 4[e]					
Ampyrone	270	670	2.5	13	8
Diflunisal	530	400	0.75	10	11
Sodium salycilate	4,956	45,000	0.92	11	10

Data from Warner TD et al. Proc Natl Acad Sci. 1999;96:7563

[a]William Harvey human whole blood assay. Radioimmunoassays of TXB_2 and PGE_2 used as a measure of COX-1 and COX-2 activities (Warner TD et al. Proc Natl Acad Sci. 1999;96:7563)

[b]IC_{80} used since relative potency of NSAID varies with concentration and NSAIDs are used therapeutically at doses that produce more than 50 % reduction of prostanoid formation

[c]Selectivities of NSAIDs toward COX-1 and COX-2 were determined by IC_{80} ratios. Higher ratios indicate increasing inhibitory (IC_{80}) amounts for COX-2 compared to COX-1

[d]Lower numbers are associated with increased selectivity toward the COX isoform

[e]For details of the four groups see Table 9.1

and human which have COX-1- and COX-2 proteins of completely different amino acid sequence to the canine COX-3 protein. COX-3 is a splice variant of COX-1. In dogs, the protein resembles the other two COX isoforms but in mice and humans it does not. Some results suggest that acetaminophen inhibits COX activity via an antioxidant action seen with phenolic compounds. At present, therefore, the utilization of COX-3 as a potential drug target in the search for analgesics does not seem worthwhile but perhaps there remains more to learn about COX isoforms and their applicability to medical science.

9.5 Sensitivities to NSAIDs

It was the progenitor drug aspirin that was the first NSAID implicated in an allergic-like reaction when, in 1902, Hirschberg reported acute

angioedema provoked by the newly marketed compound. Twenty years later, Widal, Abrani, and Lermoyez described the association of aspirin with episodes of asthma and nasal polyps, but it was another 44 years before this "aspirin triad," as it became known, was recognized and defined as a distinct clinical syndrome when Samter and Beers published their clinical studies on aspirin intolerance together with a consideration of its pathogenesis. A number of different sensitivities to NSAIDs involving different mechanisms and a wide range of symptoms from true type I anaphylaxis to delayed-type cutaneous reactions are now recognized with adverse respiratory and cutaneous responses of unknown pathogenesis in between.

9.5.1 Definition and Epidemiology

In relation to aspirin, Szczeklik and Stevenson have proposed the name "ASA [aspirin]-exacerbated respiratory disease" as the best designation of what they describe as "the aggressive and continuous inflammatory disease of the airways, combined with exacerbation of asthma and rhinitis attacks" after ingestion of aspirin and most NSAIDs. Other names commonly used are aspirin-induced asthma, aspirin sensitivity, the aspirin triad, aspirin-intolerant asthma, Widal's syndrome, and Samter's syndrome. Several names have been used to cover adverse reactions to the many different drugs collectively called NSAIDs, viz., NSAID hypersensitivity, pseudoallergy, idiosyncrasy, intolerance, and sensitivity. The two latter terms will be employed here. Depending on the symptoms and what is known of the underlying mechanisms, several types of sensitivity responses to NSAIDs have been defined (see below) but the mechanistic and associated clinical pictures are still vague enough not to be able to distinguish some cases where mixed reactions from separate categories are seen in some patients.

Sensitivity reactions occur with all NSAIDs regardless of structure, chemical and physical properties, anti-inflammatory potency, COX selectivity, and differences in mechanism that may exist (e.g., with acetaminophen). There appears to be a higher risk of anaphylaxis with aryl and heteroaryl acetic acid and propionic acid derivatives; pyrazolones may induce more immediate reactions and COX-2 inhibitors have also been implicated in sensitivity reactions. Delayed-type responses to NSAIDs with cutaneous manifestations are well recognized and although the severe necrotic epidermal reactions such as Stevens–Johnson syndrome occur, they are rare.

In the general population, aspirin sensitivity is thought to affect about 0.5–1.9 % of subjects. The incidence of aspirin sensitivity in asthmatic adults is said to be 3–5 % when history alone is considered, but aspirin challenges boost these figures up to two or threefold. It has been claimed that asthma induced by aspirin is widely underdiagnosed, and a number of surveys comparing patients with physician-diagnosed asthma with subjects without asthma appear to back this up, showing incidences of aspirin-induced asthma four to ten times higher in the former group. In one European survey, aspirin provocation tests revealed positive responses in 15 % of subjects previously unaware of their sensitivity to the drug. For patients with bronchial asthma and nasal polyps, aspirin sensitivity has been shown to be present in about one in four subjects and in chronic urticaria, an incidence of 27–35 % has been reported. Considering other NSAIDs as well as aspirin, the prevalence of sensitivity to this group of drugs is estimated to be 0.3–0.9 % of the general population, making NSAIDs the second-most common cause of drug-induced intolerance after antibiotics. NSAIDs have also been reported to be first or second on the list of drugs that provoke anaphylaxis. Patients with chronic idiopathic urticaria show an increased frequency of intolerance to aspirin and female gender, atopy, young adulthood, and intermittent use of NSAIDs are additional risk factors for NSAID intolerance.

Sensitivity to COX inhibitors is rare in children with most reactions occurring to ibuprofen, aspirin, and acetaminophen in that order. Cross-reactions between NSAIDs are frequent, but only about 10 % of NSAID-sensitive children react to acetaminophen and all of these patients react to other NSAIDs.

9.5.2 Clinical Classification of Sensitivities to NSAIDs

The natural history of aspirin-induced asthma, or aspirin triad, begins with rhinorrhea and nasal congestion. This proceeds to rhinitis which becomes perennial and associated with chronic sinusitis and nasal polyps. Asthma develops 1–5 years after the onset of rhinitis. The disease usually appears at an age of 30–34 years and its course is usually more severe and progressive in women who outnumber men.

For many investigators, the sheer number and range of clinical manifestations observed in reviewing cases of NSAID-induced sensitivities have made classification of the reactions difficult. This is understandable since symptoms may include nasal congestion, rhinorrhea, sinusitis, dyspnea, wheezing, urticaria, angioedema, and skin rashes as well as patients with a combination of respiratory and skin symptoms. In addition to the number of different clinical signs and symptoms, a consideration of the time of onset of reactions and the apparent underlying mechanisms should be undertaken before any classification of sensitivities to NSAIDs is attempted. These considerations have been taken into account in the following classifications.

9.5.2.1 NSAID-Induced Respiratory Reactions

9.5.2.1.1 Clinical Features

The classic "aspirin triad" or NSAID sensitivity consists of asthma, rhinosinusitis, and nasal polyps. Symptoms may develop stepwise over a number of years. In some patients only two of the main symptoms may be present. It is one of two cross-reactive types of NSAID sensitivity. This state of intolerance, which occurs more often in women than men, is observed in patients with aspirin-induced asthma, but the recently suggested name, aspirin-exacerbated respiratory disease, is probably a more appropriate designation for the condition that is essentially not a true drug hypersensitivity but an underlying chronic inflammatory respiratory disease occasionally exacerbated by aspirin or some other NSAID. Ingestion of aspirin or some NSAIDs by these patients provokes, within 3 h (usually 30 min to 2 h), an acute asthmatic attack that is often accompanied by rhinorrhea, inflammation of the conjunctiva, flushing, abdominal pain, and perhaps some urticaria. Most patients suffer from chronic rhinosinusitis and severe, persistent, steroid-dependent asthma with nasal polyps. Reactions to aspirin or a NSAID can precipitate an asthma attack that is severe and life-threatening.

9.5.2.1.2 Mechanisms

Although the mechanism(s) underlying these NSAID-induced respiratory reactions is not immunological, the precise cellular and molecular processes have not yet been fully defined and proven. For some years now, the most favored explanation is a pharmacologic mechanism originally proposed by Szczeklik in 1975. Asthmatic attacks were attributed to the inhibition of COX (now known to be COX-1 not COX-2) by aspirin and aspirin-like drugs in the airways of sensitive patients leading to a shift of arachidonic acid metabolism from the cyclooxygenase to the lipoxygenase pathway with an associated increased production of cysteinyl leukotrienes (Sect. 9.4.1; Figs. 9.2 and 9.3; see also Sect. 3.2.5.2 and Fig. 3.8). The consequent decreased amount of PGE_2, which normally helps to dampen the production of LTC_4, LTD_4, and LTE_4 and modulate mediator release from mast cells and other inflammatory cells, is also thought to be a contributing factor. Observations advanced to support this theory include:

1. Respiratory reactions in aspirin-induced asthma are triggered by COX-1 and not COX-2 inhibitors.
2. Airways symptoms tend to correlate with the potency of the inducing COX-1 NSAID.
3. Leukotriene receptor antagonists and inhibitors of leukotriene synthesis prevent, or partially prevent, symptoms following aspirin challenge.
4. Abnormalities in the lipoxygenase pathway may exist in patients hypersensitive to COX-1 inhibitors since they show higher levels of leukotrienes even before exposure to aspirin and other NSAIDs. Patients with aspirin-induced asthma may show increased baseline levels of urinary LTE_4 that increase after challenge with aspirin and correlate with the severity of the induced reaction.

5. Challenge with aspirin increases leukotriene levels in nasal and bronchial secretions.
6. There is some evidence that the leukotrienes are major mediators of clinical symptoms seen in asthma exacerbated by aspirin/NSAIDs, and this evidence has been bolstered by the important finding of over-expression of LTC_4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma.
7. In aspirin-sensitive rhinosinusitis, the number of cells expressing the leukotriene receptor $CysLT_1R$ (Sect. 3.2.5.2.2) is significantly higher in aspirin-sensitive than in aspirin-tolerant patients, and desensitization is associated with a decrease in the numbers of cells expressing $CysLT_1R$.
8. Japanese researchers recently demonstrated significantly higher baseline levels of LTE_4 and the abundant urinary metabolite of PGD_2, tetranor-PGDM (1,15-dioxo-9α-hydroxy-2,3,4,5-tetranorprostan-1,20-dioc acid), in patients with aspirin-induced asthma. This may be an indication of increased mast cell activation in the pathophysiology of the disease.

Genetic polymorphisms associated with NSAID intolerance and most related to enzymes in the arachidonic acid metabolic pathways have been sought but results so far have not always been consistent or led to major insights (see Sect. 9.5.5).

9.5.2.1.3 Diagnosis

Sánchez-Borges believes that the information on symptoms and exposure to NSAIDs is of the utmost importance in determining the temporal relationship between the clinical picture and the probability of a drug etiology—the diagnosis of a NSAID-induced respiratory reaction is reliable in a patient who shows repeated episodes of asthma after taking cross-reactive NSAIDs. Confirmation is obtained by oral challenge testing.

9.5.2.1.3.1 Challenge (Provocation) Tests

Although reactions to NSAIDs are said to be one of the most common drug hypersensitivities and challenge testing is currently the only sure way to diagnose or exclude true hypersensitivity, a careful evaluation involving challenges of 260 patients previously diagnosed as "hypersensitive" to NSAIDs revealed that 50 % of the patients were misdiagnosed. This result indicates that NSAID sensitivity is probably overestimated (presumably because of misinterpretation of clinical histories) and, at the same time, emphasizes the importance of challenge testing for patients suspected of sensitivity to these drugs.

Without a clear history, single or double-blinded challenge tests are obligatory. The tests are best done in a hospital environment by trained specialists (see Sect. 4.4). American and European guidelines on aspirin challenge tests have been published (see *Ann Allergy Asthma Immunol* 2007;98:172 and *Allergy* 2007;62:1111). Oral, inhalation, and nasal aspirin challenges are used and, although the sensitivities of the three tests are similar (89, 77–90, 80–86.7 %), oral challenge is generally considered to be the preferred confirmatory test for NSAIDs. Oral or inhalation challenge should not be performed in patients with unstable asthma or FEV1 % lower than 1.5 L or 70 % of the predicted value. The threshold dose of aspirin for oral challenge, that is, the smallest dose evoking a significant fall in FEV1, varies with the individual patient and may range from <10 mg to 600 mg. For oral challenge with aspirin, placebo and drug are given at one and a half to 2 h intervals, and FEV1 is measured at baseline and then every 30 min. On day one, placebo is given in four doses; on day two, four increasing doses of aspirin are given until a maximum single dose of 500 mg is reached or a clinical reaction results. Other protocols recommend a maximum dose of aspirin of 325 mg. A positive reaction is the appearance of clinical symptoms or a decrease in FEV1 equal to or greater than 20 % of the baseline figure. For inhalation challenge, lysine–aspirin is administered in increasing doses using a dosimeter-controlled nebulizer. Doses are given every 30 min and FEV1 measurements are taken every 10 min after each drug administration. A positive response is again the appearance of symptoms or a 20 % fall in FEV1, the latter obtained from construction of a dose response curve. For patients with severe asthma, nasal challenge with lysine-aspirin can be considered. Nasal symptoms are evaluated with the aid of peak nasal inspiratory flow, acoustic rhinometry, or active anterior rhinomanometry.

9.5.2.1.3.2 Basophil Activation Test

Because of the occasional presence of mixed symptoms, blurred demarcation lines between classified NSAID sensitivities and uncertainty about the putative mechanism(s) underling the reactions, there is at present an absence of obvious in vitro complementary diagnostic tests for the different NSAID-induced responses. The basophil activation test (BAT) has been applied to the diagnosis of reactions and its use advocated, but there is no common agreement about the test's general usefulness or even applicability. Only a minority of hypersensitive reactions to NSAIDs are IgE antibody-mediated with most of the reactions being respiratory and cutaneous responses apparently resulting from drug-induced inhibition of COX-1, depletion of PGE_2, and a resultant increase in production of cysteinyl leukotrienes and other mast cell mediators. When BAT has been employed with suspected cases of NSAID sensitivity, the participating patients have not always formed a clinically homogeneous group sometimes with mixed respiratory, cutaneous, immediate, and delayed reactions among the selected cases. Despite this, a number of studies from Spain claim that basophil activation induced by aspirin and other NSAIDs is useful for the in vitro diagnosis of what has sometimes been termed the "NSAID hypersensitivity syndrome" (see below, Sect. 9.5.4).

9.5.2.1.3.3 Test for Release of Cysteinyl Leukotrioenes

The Cellular Allergy Stimulation Test (CAST®), designed to measure the release of cysteinyl leukotrienes from leukocytes following allergen challenge (see Sect. 4.5.3.1), has been applied as an ELISA or combined with flow cytometry in the Flow CAST® for the in vitro diagnosis of aspirin- and other NSAID-induced sensitivity. In a Polish investigation, application of the CAST to aspirin-intolerant and tolerant asthmatics after stimulation with lysine–aspirin revealed a weak stimulatory effect on leukotriene release in both groups of patients. The verdict of the investigators was that the test had no value for diagnosis of aspirin-induced asthma. In their continuing application of the CAST assay, the de Weck/Sanz group in Pamplona studied 60 "aspirin- and/or NSAID-hypersensitive" patients. A flow cytometric BAT for aspirin showed sensitivity of 42 % and a specificity of 100 %. Sensitivities with other NSAIDs were not impressive ranging from 15 % for metamizol to 55 % for naproxen. Adding the CAST results to the sensitivity finding for all four NSAIDs tested, increased the sensitivity to 73 % with a specificity of only 71 % making the conclusion that the basophil test might "help avoid some cumbersome and dangerous provocation challenges" debatable. In at least two other investigations of cysteinyl-leukotriene release in aspirin-induced asthmatics, higher amounts of the liberated mediators in the aspirin-sensitive patients were recorded, but the low sensitivity and predictive values were seen as limiting the clinical usefulness of the test in the diagnosis of aspirin sensitivity.

9.5.2.1.3.4 Generation of 15-HETE from Peripheral Blood Leukocytes

Proceeding from the observation that aspirin specifically triggers the release of 15-hydroxyeicosatetraenoic acid (15-HETE) (Fig. 9.3; Sect. 3.2.5.2 and Fig. 3.8) from epithelial cells of nasal polyps and peripheral blood leukocytes from patients with aspirin-induced asthma/rhinosinusitis, 15-HETE generation was utilized as a test for the identification of aspirin-sensitive patients. Stimulation in vitro of peripheral blood leukocytes with 200 μM of aspirin resulted in a mean increase of over 400 % in 15-HETE generation but only small to insignificant responses were seen in aspirin-tolerant asthmatic and control subjects. Sensitivity of the test was 83 % and specificity 82 %; positive and negative predictive values were 0.79 and 0.86, respectively. The NSAID COX-1 inhibitor naproxen also triggered 15-HETE release but COX-2-selective NSAIDs did not.

9.5.2.1.4 Patient Management

Patients who experience exacerbation of their asthma after taking aspirin or other NSAID COX-1 inhibitors should avoid these drugs as well as drugs that increase leukotriene levels and topical or systemic corticosteroids. NSAIDs that have only weak inhibitory activity for the ultimate synthesis of prostaglandins such as acetaminophen and selective COX-2 inhibitors seem to be tolerated by most patients with aspirin-induced

Table 9.3 Oral desensitization protocol for patients with aspirin-induced asthma (aspirin-exacerbated respiratory disease)

Time (Hours)	Placebo or aspirin dose (mg) on		
	Day 1	Day 2	Day 3
0	Placebo	30	150
3	Placebo	60	325
6	Placebo	120	650

Following successful desensitization, daily aspirin tablet treatment is maintained

Adapted from Szczeklik A, Stevenson DD. J Allergy Clin Immunol. 1999;104:5

asthma. Caution should be exercised, however, with high doses of acetaminophen and preferential COX-2 inhibitors like nimesulide and meloxicam since loss of tolerance and sensitivity reactions may result. Oral tolerance tests with these drugs are therefore recommended before the drugs are employed for regular use.

9.5.2.1.5 Desensitization

Tolerance to aspirin (or other NSAIDs) can be induced by repeated oral administrations of drug, but to maintain tolerance, or desensitization, the patient needs to ingest the drug on a regular (usually daily) basis. The "Scripps Clinic Protocol," devised by Szczeklik and Stevenson for oral desensitization of patients with aspirin-induced asthma is based on the administration of small incremental doses of aspirin over a 3 day period until 400–650 mg of the drug is tolerated (Table 9.3). After successful desensitization, daily aspirin treatment is maintained. Szczeklik and Stevenson recommend that aspirin desensitization followed by a daily maintenance dose be considered for aspirin-induced asthma patients whose disease is controlled only with unacceptably high doses of corticosteroids, patients who require repeated nasal polypectomies or sinus surgery, and those who need aspirin or other NSAIDs for treatment of, for example, their coronary artery disease, thromoembolism, etc.

9.5.2.2 NSAID-Induced Cutaneous Reactions

Although urticaria and angioedema may be seen as minor symptoms in about 5 % of patients with aspirin-induced asthma and during some aspirin challenges, cutaneous reactions make up a major component of symptoms seen in patients with the following clinical patterns:

9.5.2.2.1 Cross-Reacting NSAID-Induced Urticaria and Angioedema

For an extended discussion of urticaria and angioedema see Sect. 3.2.8.

In patients with asthma or chronic urticaria, the incidence of NSAID sensitivity is said to be 23–28 %. Some patients with chronic idiopathic urticaria develop wheals and angioedema or an increase in urticaria after receiving aspirin. Such reactions generally develop 1–4 h after administration of the drug although the time may be as short as 15 min and as long as 24 h. Reactions usually resolve within a few hours but may continue for up to 10 days. In contrast to aspirin-induced asthma, elucidation of the mechanisms involved in aspirin-induced urticaria/angioedema has been a slow process. As well as aspirin, NSAIDs, like ibuprofen, naproxen, and diclofenac that inhibit COX-1, may cause an increase in wheals and swelling in up to one-third of patients with chronic urticaria. In an evaluation of cross-sensitivity between aspirin and selective COX-2 inhibitors in chronic idiopathic urticaria patients, Szczeklik and collaborators found that rofecoxib (37.5 mg) and celecoxib (300 mg) did not elicit skin eruptions in any aspirin-sensitive patients (see also below under Management) indicating that there is no cross-reaction between these two COX-2 inhibitors and aspirin, and aspirin-induced urticaria-angioedema is a COX-1-dependent process. Caution should be exercised, however, when considering the use of COX-2 inhibitors in patients with NSAID-induced urticaria and angioedema. Some recent results with etoricoxib suggest that COX-2 inhibitors may not be safe in such patients who are intolerant to a number of NSAIDs including acetaminophen.

Urinary LTE_4 measurements indicate that aspirin-sensitive chronic idiopathic urticaria is associated with overproduction of cysteinyl leukotrienes. NSAID sensitivity in urticaria is reflected in the overproduction of cysteinyl leukotrienes including basal production of LTE_4. Inhibition of COX-1 and alterations in eicosanoid production, including an increase in PGD_2 levels,

following aspirin challenge in patients with urticaria and angioedema, suggests a similar mechanism to NSAID-induced asthma with release of mediators from inflammatory cells in the skin. This was confirmed by the detection of significant increases in urinary LTE_4 and the stable metabolite of PGD_2, 9α, 11β-PGF_2 in patients' plasma. Patients with chronic urticaria and NSAID sensitivity show a higher incidence of asthma and nasal polyps compared to those with chronic urticaria alone.

For diagnosis, skin testing with aspirin and other NSAIDs is not indicated and in the absence of proven, validated, and standardized in vitro tests, oral challenge is currently the only way to diagnose or exclude NSAID sensitivity. Even so, the sensitivity of challenge testing is not 100 % and false negative results occur. Oral provocation should not be performed during periods of active urticaria; a single-blind placebo-controlled protocol should be employed at least 1–2 weeks after skin reactions, and testing should be undertaken over 2 days. After four separate doses of the placebo on day one, four increasing doses of 71, 117, 312, and 500 mg (total 1,000 mg) of aspirin are administered at intervals of one and a half to two hours. Testing is interrupted if symptoms of sensitivity to NSAIDs appear. Most challenged patients react to an aspirin dose of 325–650 mg. For challenge testing with other NSAIDs, see Chap. 4, Table 4.3.

9.5.2.2.1.1 Management

In the management of patients with cutaneous sensitivity to NSAIDs, avoidance measures are similar to those employed for aspirin-/other NSAID-induced asthma, viz., avoid selective COX-1 inhibitors but acetaminophen is generally well tolerated. The selective COX-2 inhibitors etoricoxib, celecoxib, and rofecoxib appear to be well tolerated; however, in one assessment of the tolerability of some COX-2 inhibitors, reactions to the latter two drugs were seen in 33 % and 3 % percent of patients, respectively, indicating that controlled oral provocation testing should still be performed. The importance of provocation testing is also highlighted by the intolerance of etoricoxib in cross-reactive patients intolerant to acetaminophen.

Table 9.4 Oral desensitization protocols for patients with aspirin/NSAID-induced cutaneous reactions

Protocol	Time	Placebo or aspirin dose (mg)
Wong et al[a]	0 min	0.1
	15 min	0.3
	30 min	10
	45 min	30
	60 min	40
	85 min	81
	110 min	162
	135 min	325
Schaefer-Gore[b]	0 h	Placebo
	1 h	150
	2 h	325
	3 h	Placebo
	4 h	325
	5 h	Placebo
	6 h	END

Patients may be maintained on aspirin 81 mg daily if necessary after careful assessment

Note: These protocols to be used only if there is zero-to-minimum risk of a respiratory or anaphylactoid reaction

[a]Data from Wong JT et al. J Allergy Clin Immunol. 2000;105:997

[b]Data from Schaefer OP, Gore JM. Cardiology. 1999;91:8

9.5.2.2.1.2 Desensitization

Although desensitization of patients with aspirin-induced urticaria-angioedema has been said to be difficult to achieve and maintain, successful oral protocols, both rapid and long, have been published. To shorten the previously published desensitization procedures involving dosage intervals of 2–24 h, a starting dose of 0.1 mg was selected, and this was increased 3–3.3-fold in early steps and twofold in later steps at 10–30 min intervals allowing the whole procedure to be completed within a few hours (Table 9.4). In a study involving 11 patients with histories of aspirin- or NSAID-induced urticaria or angioedema, nine patients tolerated the procedure without adverse effects and continued taking aspirin for from 1 to 24 months without developing urticaria or angioedema. The two intolerant patients had urticaria due to other agents as well as NSAIDs. In some other published protocols, the maximum dose of aspirin administered is 325 mg (Table 9.4)

9.5.2.2.2 Multiple NSAID-Induced Urticaria and Angioedema

Note that in a recent "update," Sánchez-Borges et al. (Pharmaceuticals. 2010;3:10) include anaphylaxis along with urticaria and angioedema as one of the manifestations of this intolerance.

This variant of NSAID-induced intolerance is more prevalent in atopics and occurs in otherwise normal, healthy cross-reactive subjects with acute urticaria and/or angioedema but with no other underlying skin or respiratory disease. Facial angioedema, occurring within minutes or up to 24 h after ingestion of an NSAID, is the most frequent clinical manifestation. The mechanism(s) of these reactions has not been elucidated but the most likely mechanism may be related to COX-1 inhibition as is the case with cross-reactive NSAID sensitivities. IgE antibodies do not seem to be involved because of the variety of different NSAID structures involved. For diagnosis, skin tests and in vitro tests are not considered relevant, so diagnosis tends to be on the basis of reactions to more than one NSAID in patients who are otherwise healthy. Up to 80 % of patients with sensitivity to a number of NSAIDs are said to tolerate acetaminophen and nimesulide but tolerance of COX-2 inhibitors varies, and these drugs may induce reactions in some NSAID cross-reactive patients. The capacity to induce reactions may depend on the relative COX-1 inhibitory activities of the COX-2 drugs, for example, nimesulide, meloxicam, and celecoxib each possess some COX-1 inhibitory activity while selective COX-2 inhibitors rofecoxob and valdecoxib appear to be well tolerated by the majority of NSAID-sensitive patients with cutaneous reactions. However, after studying a case of rofecoxib-induced urticaria and angioedema in an aspirin-sensitive patient, Ring and collaborators suggested that COX-2 inhibition may play a role in the pathogenesis of urticaria and they concluded that use of a selective COX-2 inhibitor in aspirin-sensitive patients is no guarantee of safety. Controlled challenges are recommended before administration of any of the so-called specific COX-2 inhibitors to NSAID-sensitive patients. A typical oral provocation protocol (for aspirin) involves two placebo doses given 2 h apart on day one, two doses of drug, 100 mg and 200 mg, given 2 h apart on day two, one dose of 325 mg on day three, and 650 mg on day four. Skin scores are recorded every 2 h.

Successful desensitization of otherwise healthy individuals with multiple NSAID-induced urticaria and angioedema but no underlying skin disorder does not appear to have been reported.

9.5.2.2.3 Single NSAID-Induced Urticaria, Angioedema, and/or Anaphylaxis

These reactions, said to constitute about 30 % of all cases of NSAID sensitivity, are induced by a single or chemically closely related NSAID and may occur more often in subjects who are atopic, female, and have a history of food or drug allergy. Reactions occur to pyrazolones in particular but also to diclofenac and less often to acetaminophen, aspirin, ibuprofen, indomethacin, ketorolac, sulindac, fenoprofen, tolmetin, meclofenamate, naproxen, piroxicam, and celecoxib. Clinical manifestations include urticaria, angioedema, laryngeal edema, generalized pruritus, rhinitis, bronchospasm, and anaphylaxis. Urticaria/angioedema generally occurs within minutes of oral or IV exposure, and anaphylactic shock and death have been reported. Sensitivity to a single NSAID with tolerance of other chemically unrelated NSAIDs indicates that a COX-1-related mechanism for these reactions is unlikely but the timing and characteristics of the symptoms points to an IgE antibody-mediated mechanism. Evidence for this is strongest with the pyrazolone drugs where positive skin tests and drug-reactive IgE antibodies have been detected.

9.5.2.2.3.1 Sensitivity to Pyrazolones

There are two forms of pyrazolone sensitivity, both apparently non-dose-related—reactions resembling aspirin-induced asthma, probably involving inhibition of prostanoid synthesis and overproduction of cysteinyl leukotrienes and immune type I immediate reactions manifesting as urticaria, angioedema, and anaphylaxis. Reports of anaphylactic shock reactions

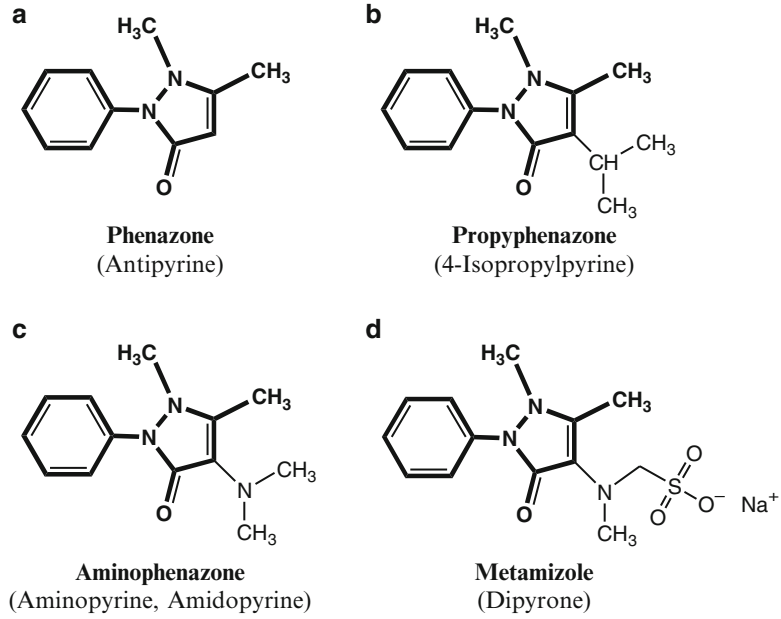

Fig. 9.4 Some important pyrazolone nonsteroidal anti-inflammatory drugs showing the 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one common core structure in *bold*

following administration of aminophenazone (aminopyrine; amidopyrine) and metamizol (dipyrone) date back over 40 years but there is some evidence that such reactions to pyrazolones (including the above two drugs, phenazone and propyphenazone) (Fig. 9.4), were observed much earlier. IgE antibodies to propyphenazone, well known for occasional severe adverse reactions, were detected in a study of patients with symptoms suggestive of immediate allergic reaction to the drug. After linking a spacer arm to *N*-demethylpropyphenazone and conjugating to human serum albumin with carbodiimide (EDC)/S-*N*-hydroxysuccinamide, the drug conjugate was employed to detect specific IgE antibodies in an ELISA system alongside skin tests with propyphenazone 0.25 %. Positive wheal and flare skin test reactions were seen in 44 of the 53 patients (83 %) and propyphenazone-specific IgE antibodies were detected in 58 % of the patients, but seven of nine skin test-negative patients also had propyphenazone-reactive IgE antibodies. Inhibition of IgE binding to the drug conjugate by propyphenazone but not by phenazone, aminophenazone, or metamizol showed that the IgE antibody assay was specific for propyphenazone, demonstrating lack of cross-reactivity between the different pyrazolone drugs, a point to be borne in mind for skin testing anaphylactic patients with the culprit drug. A solid phase immunoassay for demonstrating the presence of IgE antibodies to pyrazolone drugs has also been developed and applied for the detection of the 1-phenyl-2,3-dimethyl-3-pyrazoline-5-one common core structure found in aminophenazone, propyphenazone, and metamizol as well as the "parent" drug phenazone (Fig. 9.4). A strong association between pyrazolone drug hypersensitivity and HLA-DQ and DR antigens has been reported.

Of other NSAIDs, those more often implicated in single NSAID-induced anaphylactic reactions include diclofenac, acetaminophen, ibuprofen, and naproxen. Skin testing of diclofenac hypersensitive patients has persistently given negative results leading investigators to conclude that a drug antigenic determinant(s) results from protein binding of a metabolite(s) generated

in vivo. The nature of the putative metabolite(s) is uncertain but 5-hydroxydiclofenac and auto-oxidation products 5-hydroxydiclofenac quinoneimine and 4 -hydroxydiclofenac quinoneimine have been advanced as possible antigenic determinants following experiments in mice. However, experiments with human serum albumin conjugates of diclofenac and its 3-, 4-, and 5-OH phase I metabolites did not detect any patients with specific IgE antibodies to the drug or its metabolites in sera from 59 patients with a history of acute hypersensitivity to diclofenac. In addition, BAT and CAST assays with representative patient samples detected no upregulation of CD63 with BAT and no significant production of cysteinyl leukotrienes with CAST. The overall conclusion was that there was no evidence for an IgE-mediated mechanism based on hapten–protein conjugates in diclofenac hypersensitive patients, and the involvement of the most relevant metabolites of the drug could be excluded. There appears to be about 40–50 cases of anaphylactic reactions to acetaminophen. Oral challenge is the most successful diagnostic method applied, there are only a few reports of positive skin tests to the drug, and at least one report of the detection of specific IgE antibodies. Females predominate in reported reactions. In a few cases, the need to dose beyond a threshold (e.g., 100 mg in one patient) to induce an anaphylactic reaction has been observed. Collective data on the risks of different NSAIDs is hard to obtain but, between 1,985 and 2,000, The Netherlands Pharmacovigilance Foundation received reports of 76 cases of NSAID-induced anaphylaxis with naproxen, ibuprofen, and diclofenac disproportionately represented. The results strengthen previous findings of a higher risk of anaphylaxis associated with these three drugs compared to other NSAIDs.

For reliable skin test diagnosis, testing should be undertaken as soon as possible after the reaction as a loss of sensitivity with time is apparent. To establish the safety of alternative drugs, oral challenges are necessary, but oral challenge testing with the culprit drug and structurally similar drugs should be assessed against the risks involved. Application of BAT using CD63 expression as marker in two studies of patients with hypersensitivity to the pyrazolone, metamizol, an analgesic known to cause IgE-mediated immediate reactions, showed sensitivities of 55 and 42 % and specificities of 86 and 100 %, respectively. In the latter study, CAST® had a sensitivity of 52 % with a specificity of 90 % and when considered along with the BAT and skin test findings, the three tests together identified 77 % of the drug-allergic patients. Both investigations found a time-dependent decrease in BAT- positive reactions with approximately 60 % of patients becoming negative after 6 months.

For management of the patient, strict avoidance of the culprit drug and cross-reactive drugs (unless shown to be tolerated) is necessary, and alternative NSAIDs should only be administered if first shown to be tolerated by challenge testing.

9.5.2.3 Delayed Reactions to NSAIDs

These are responses that generally develop more than 24 h after exposure to a NSAID and which appear to be immune-based type IV reactions mediated by T cells. Table 9.5 lists cutaneous and/or systemic reactions that may be provoked by NSAIDs together with the drugs most commonly implicated. Diagnosis is based on the clinical picture of symptoms and their recurrence, appearance, and location of skin lesions; involvement of other organs; and, of course, temporal relationships. Patch tests and the lymphocyte transformation test may be employed, although the sensitivity and specificity of the former is likely to be unknown and/or in question and the validation, reliability, and cost of the latter method are considerations (see Chap. 4). Again, drug provocation testing is the gold standard although it is, of course, contraindicated in severe, generalized reactions (Chap. 4). In the management of delayed-type reactions, early withdrawal of the offending NSAID is essential and systemic treatment with corticosteroids and antihistamines may be warranted. Combined immediate- and delayed-type hypersensitivity to NSAIDs may occur indicating that patch as well as prick and intradermal testing should be kept in

Table 9.5 Delayed reactions to NSAIDs. Clinical manifestations and drugs implicated

Clinical manifestations	Drugs commonly implicated	Comments
Maculopapular eruptions	Ibuprofen, flurbiprofen, diclofenac, pyrazolones, celecoxib	Probably specific T cell hypersensitivities
Fixed drug eruptions (FDE)	Aspirin, ibuprofen, naproxen, indomethacin, diclofenac, diflunisal, mefenamic acid, piroxicam, phenylbutazone, pyrazolones, acetaminophen, nimesulide	NSAIDs are among the most common causes of FDE Cross reactions may occur
Contact and photo-contact dermatitis	Diclofenac, indomethacin, ibuprofen, flurbiprofen, ketoprofen, flufenamic acid, etofenamate, bufexamac, tiaprofenic acid	Topical ketoprofen frequently implicated Chemically related cross-reactivity observed Patients sensitized topically may develop cutaneous reactions to same drug given orally or parenterally
Acute generalized exanthematous pustulosis (AGEP)	Ibuprofen, nimesulide, etoricoxib, valdecoxib, celecoxib, aspirin, acetaminophen	Reactions rare
Drug reaction (rash) with eosinophilia and systemic symptoms (DRESS)	Oxicams, celecoxib, ibuprofen	Reactions rare
Severe bullous cutaneous reactions (SJS, TEN)	Oxicams, phenylbutazone, oxyphenylbutazone, some COX-2 inhibitors, diflunisal	Reactions rare Symptoms may occur up to 8 weeks after drug administration
Pneumonitis	Naproxen, sulindac, fenbufen, ibuprofen, tolfenamic acid, diclofenac, phenylbutazone, oxyphenylbutazone	Symptoms of cough, fever, dyspnea, malaise. Typically responds to drug withdrawal but corticosteroids may be needed. Exact etiology not known
Nephritis	Indomethacin, sulindac, ibuprofen, tolmetin, piroxicam, rofecoxib, valdecoxib, celecoxib	Symptoms—rash, fever, eosinophilia or eosinophiluria, acute renal failure Progression of disease may depend on balance between relative strengths of immune reaction and anti-inflammatory effects of drug
Aseptic meningitis	Ibuprofen, sulindac, naproxen, tolmetin, ketoprofen, diclofenac, indomethacin, piroxicam, rofecoxib, celecoxib	Rare. No obvious cross-reactivity of NSAIDs. Mechanism may be type III or IV hypersensitivity to drug

SJS Stevens–Johnson syndrome, *TEN* toxic epidermal necrolysis

mind in assessing some patients. Patients with severe bullous cutaneous reactions such as Stevens–Johnson syndrome and toxic epidermal necrolysis are treated as for burns victims in intensive care.

9.5.2.4 Mixed and Systemic Patterns of NSAID-Induced Sensitivity

Illustrating how difficult it can sometimes be to classify a patient who has reacted adversely to an NSAID into one of the three main clinical patterns presented here, viz., respiratory, cutaneous (with three further sub-patterns each involving urticaria/angioedema), and delayed, Sánchez-Borges has categorized so-called non-immediate allergic and pseudoallergic adverse reactions to NSAIDs into four main patterns—respiratory, cutaneous (again with three sub-patterns manifesting urticaria/angioedema), mixed, and systemic.

9.5.2.4.1 Mixed Pattern

The mixed pattern, said to be seen in about 30 % of NSAID-sensitive patients during controlled challenge, is a blend of respiratory and cutaneous

symptoms that include cough, breathlessness, hoarseness, rhinorrhea, wheezing conjunctival itch, urticaria, and angioedema. Absence of clear signs and symptoms of chronic severe asthma, sinusitis, nasal polyps, chronic urticaria, and anaphylaxis is another indication that these patients need to be categorized into a separate group.

9.5.2.4.2 Systemic Pattern

In this category, reactions may include flushing, nasal and ocular symptoms, bronchospasm, urticaria, abdominal pain, and, occasionally, vasomotor collapse. The reactions may be anaphylactoid or anaphylactic, the latter usually observed in patients who reacted to a single NSAID while tolerating other chemically unrelated NSAIDs. The involvement of drug-reactive IgE antibodies is shown by positive prick or intradermal tests and, if available, immunoassays for drug-specific IgE and/or BAT. Some surveys have concluded that NSAIDs constitute the second largest group of drugs responsible for anaphylactic reactions. In addition to the pyrazolones, there are case reports of anaphylactic reactions to just about every known NSAID such as aspirin (see below), ibuprofen, diclofenac, indomethacin, naproxen, fenoprofen, sulindac, zomepirac, piroxicam, meclofenamate, tolmetin, ketorolac, glafenine, acetaminophen, and a number of coxibs including celecoxib, valdecoxib, and etoricoxib.

After reviewing the classification of the various intolerant states provoked by NSAIDs, two specific aspects pertaining to mechanisms of action and diagnosis of hypersensitive responses to these drugs, namely, IgE antibody responses to aspirin and application of BAT to diagnosis, are considered. The subject of serum IgE antibodies to aspirin is considered separately since they may be found not only in patients classified into different sensitivity categories but also in patients where more than one mechanism is implicated. As a diagnostic method for NSAID intolerances, BAT has been applied to patients with a heterogeneous mix of symptoms, some of which are associated with IgE-independent respiratory and cutaneous reactions. Many of these patients seem inappropriate candidates for the test.

9.5.3 IgE Antibodies to Aspirin

In 1981, a new medical graduate beginning a Ph.D in the authors' laboratory, was disturbed by the plight of an unusually high number of aspirin-sensitive patients with chronic urticaria attending the weekly allergy clinic. Thinking that, in at least some of the patients, there was a high probability of an underlying IgE-mediated mechanism for the distressing reactions, a strategy was devised to examine the patients' sera for the presence or absence of such antibodies. Nineteen patients, mainly non-atopic with chronic urticaria were tested for specific anti-salicyloyl serum IgE antibodies using a freshly prepared salicyloyl-polylysine solid phase conjugate. With polylysine succinate as control, skin prick tests and in vitro histamine release were also investigated using the aspirin-polylysine antigen. No positive responses were obtained in any of the tests leading to the conclusion that the clinical symptoms and signs in patients with chronic urticaria associated with aspirin sensitivity are not mediated by specific IgE antibodies. In view of what has been learned of the classification and mechanisms involved in NSAID sensitivities, it seems likely that the 19 patients studied over 30 years ago would today be placed in the cutaneous pattern group in the classification of NSAID-sensitive patients. The salicyloyl determinant and the *O*-methylsalicyloyl derivative, each linked to a solid phase by a spacer arm, were later employed by Zhu in Beijing to look for aspirin-specific IgE antibodies in the sera of 28 patients with positive histories of aspirin sensitivity, most confirmed by oral challenge. Ten patients had asthma, 16 urticaria/angioedema, one generalized flushing with pruritus, and one rhinoconjunctivitis. The sera of 27 (96.4 %) and 20 (71.4 %) patients were shown to have salicyloyl- and *O*-methylsalicyloyl-specific IgE antibodies. The former antibodies were strongly inhibited by salicylic acid and good inhibition was also seen with 2-aminophenol, indicating antibody recognition of the hydroxy group ortho to the attached spacer group. *O*-Methylsalicylic acid also proved to be a good inhibitor of its complementary antibodies as was

indomethacin which was weaker but still surprisingly active. At the clinical level, indomethacin in both low and therapeutic doses has been shown to elicit positive provocation reactions in aspirin-sensitive asthmatics and cross-desensitization with the two drugs has been reported. This cross-recognition seen both in vitro and in vivo seems to have its basis in the structural similarity between *O*-methylsalicylic and the *O*-methylphenol structure that is part of the indole nucleus of indomethacin.

Starting with a population of more than 100 patients suspected of aspirin intolerance and suffering from asthma with or without nasal polyposis, Sainte-Laudy in Paris employed the clinical histories, skin tests, IgE determinations, and BAT to find nine cases involving an IgE antibody-dependent mechanism. For the IgE assay, aspirin–lysine was mixed with epoxy (bis-oxirane)-activated Sepharose (Sect. 4.3.1) to form the aspirin solid phase.

9.5.4 NSAID Sensitivities and BAT

In a flow cytometric determination of basophil activation induced by aspirin and other NSAIDs, leukocytes from 60 NSAID sensitive patients (38 with cutaneous, 20 with airway, and 2 with cutaneous and airway symptoms) were stimulated with aspirin, acetaminophen, metamizol, diclofenac, and naproxen. Sensitivities and specificities, respectively, obtained with the different drugs were: aspirin 43 and 100 %; acetaminophen 12 and 100 %; metamizol 15 and 100 %; diclofenac 43 and 93 %; naproxen 55 and 74 %. These results led the investigators to conclude that the test might help to avoid some challenge tests with NSAIDs. In a later extension of this in vitro diagnostic investigation of what was termed the NSAID "hypersensitivity syndrome," a multicenter study performed within the framework of the European Network for Drug Allergy (ENDA) found 57 % of 140 patients were BAT-positive to multiple NSAIDs, 27 % were positive with only one or two concentrations of a single NSAID, and 16 % were negative. Of the patients described as having a history of hypersensitivity to NSAIDs, 27 % had airway symptoms, 67 % had skin symptoms of urticaria/angioedema, and 6 % presented with both. Given the mixed symptom patterns and what is currently understood of the mechanisms underlying the variously classified sensitivities to NSAIDs, the high proportion of positive BAT responses found in this study is surprising. Again, it was concluded that BAT would be particularly appropriate for patients with a clinical history of NSAID intolerance but in whom challenge tests are not advisable. In what seems to be a more understandable application of BAT, viz., employment with cells from patients with immediate reactions (anaphylaxis, urticaria, angioedema, asthma, conjunctivitis) to one or more NSAIDs, results of a study on 43 patients with the NSAIDs aspirin, ibuprofen, metamizol, diclofenac, acetaminophen, and ketorolac showed a sensitivity of 43 %, specificity of 100 %, and positive and negative predictive values of 100 % and 54 %, respectively. Two other BAT-based diagnostic investigations, however, one examining different NSAIDs and the other looking only at diclofenac, concluded that the value of the test in NSAID intolerances is yet to be firmly established. Interestingly, although diclofenac induced basophil degranulation, upregulation of the CD63 marker antigen did not occur in NSAID-sensitive patients.

9.5.5 Genetic Mechanisms of Aspirin-Induced Sensitivities

A study in a polish population of possible genetic markers for aspirin-induced asthma revealed an association between HLA-DPB1*0301 and this phenotype. This result was later confirmed in a Korean population by H-S Park and coworkers where it was found that patients with DPB1*0301 were more often females with a lower FEV1 and a higher incidence of rhinosinusitis and nasal polyps than those lacking this marker. Enhanced expression of LTC_4 synthase due to overactive transcription of an allelic variant (LTC_4S-444A>C) associated with aspirin-induced asthma has also been reported from Poland. Patients with the C allele had a greater risk of

developing aspirin-induced asthma, and those homozygous for LTC_4S-444A had higher increases in urinary LTE_4 after aspirin challenge. Note, however, that this association has not been observed in some other populations including American, Japanese, and Korean. A suggested possible involvement of the 5-lipoxygenase gene (ALOX5) in aspirin-induced asthma has been supported by the detection of a significant association between ALOX5 promoter polymorphism and the severity of airway hyperresponsiveness in a Korean population. Genetic associations between the leukotriene receptor genes CYSLTR1 and CYSLTR2 and aspirin-induced asthma were also established in Park's laboratory where three single nucleotide polymorphisms (SNPs) in the promotor region of CYSLTR1 were significantly associated with the asthma phenotype, especially in males. A functional SNP of the PGE_2 subtype 2 gene, PTGER2, and two SNPs of TBXA2R were also found to be associated with aspirin-induced asthma.

A strong positive association has been shown between aspirin-induced urticaria/angioedema and the HLA DRB1*1302 and HLA DQB1*0609 alleles, and it has been suggested that HLA-DRB1*1302-DQB1*0609-DPB1*0201 may be a genetic marker for determining this phenotype. Promoter polymorphisms of ALOX5 were significantly different between aspirin-induced urticaria/angioedema and aspirin-induced asthma and an FcεRIα gene promoter polymorphism was significantly associated with the former phenotype.

It is hoped that further genetic studies will provide more insights into the molecular genetic mechanisms of aspirin-induced sensitivities and find reliable genetic markers for predicting drug responses. This, in turn, might lead to improved diagnostic approaches and therapy.

Summary

- NSAIDs are one of the most widely and commonly used drugs worldwide and are responsible for about 20–25 % of adverse drug reactions. About 30 % of adults in the USA take over-the-counter NSAIDs on a regular basis for pain.
- Ibuprofen and diclofenac are the most commonly consumed NSAIDs. Naproxen, meloxicab, celecoxib, ketoprofen, and etorocoxib are also high on the list.
- Many NSAIDs are organic acids that bind to plasma proteins. Different chemical groups are recognized: salicylates, propionic acid derivatives, aryl and heteroaryl acetic acids, anthranilates (fenamic acid derivatives), oxicams (enolic acids), phenylpyrazolones, analides, COX-2-selective inhibitors.
- Prostaglandin endoperoxide H synthases (PGHS-1, PGHS-2; cyclooxygenases COX-1, COX-2) catalyze the formation of prostaglandin G_2 (PGG_2) from arachidonic acid. PGG_2 is then reduced to PGH_2 which is converted by specific synthases to PGD_2, PGE_2, $PGF_2\alpha$, prostacyclin PGI_2 (a vasodilator), and thromboxane A_2, TXA_2 (a vasoconstrictor).
- Inhibition of COX-1 reduces production of protective PGE_2 and PGI_2 in the stomach, which may lead to gastric ulceration.
- COX-2, expressed only in inflammation, is induced by the cytokines IL-1 and TNF and by LPS. Selective COX-2 inhibitors show fewer gastrointestinal side effects but can produce cardiovascular effects, probably by suppressing cardio-protective PGI_2.
- NSAIDs can be classified on the basis of their COX inhibitory and selective properties. Most NSAIDs inhibit both COX-1 and COX-2 to some extent. Most are mainly COX-1 inhibitory, e.g., aspirin, ibuprofen, diclofenac, naproxen, while some are COX-2 inhibitory, e.g., celecoxib, rofecoxib, and lumiracoxib.
- Vane et al. established the relative inhibitory potencies of NSAID COX-1 and COX-2 inhibitors. NSAIDs with the highest gastrointestinal toxicity have the highest COX-1 selectivity.
- Discovered in 2002, the so-called COX-3 isoenzyme, inhibited by acetaminophen, phenacetin, and metamizole, resembles the other two isoforms in dogs but not in humans or mice. COX-3 is, in fact, a splice variant of COX-1.

- In sensitive subjects, aspirin (and other NSAIDs) is associated with the so-called "aspirin triad" or aspirin-induced asthma (AIA; also called aspirin-exacerbated respiratory disease). Classic AIA consists of chronic asthma and rhinosinusitis together with nasal polyps. The incidence of AIA in adult asthmatics is about 3–5 %.
- Aspirin together with other NSAIDs form the second biggest group causing drug-induced intolerance after antibiotics.
- The mechanism of NSAID-induced respiratory reactions appears to be due to the redirection of arachidonic acid metabolism from the COX to the lipoxygenase synthetic pathway with associated production of cysteinyl leukotrienes. PGE_2 normally helps to dampen the production of the leukotrienes.
- Challenge testing is the only sure way to diagnose or exclude true sensitivity to an NSAID. Oral challenge with drug and placebo is usually undertaken over a 2 day period. A maximum dose of 325 mg or 500 mg aspirin is often used.
- Other diagnostic methods sometimes employed include BAT (often of doubtful value) and measurement of released cysteinyl leukotrienes and 15-HETE.
- Desensitization can be induced by repeated oral administration of drug, e.g., the "Scripp's Protocol." To maintain tolerance, patients need a daily dose of drug.
- NSAID-induced cutaneous reactions occur in a number of different clinical patterns—cross-reacting NSAID-induced urticaria and angioedema; multiple NSAID-induced urticaria and angioedema; single NSAID-induced urticaria and angioedema or anaphylaxis.
- Reactions to pyrazolone drugs can resemble AIA or be type I IgE-mediated hypersensitivity.
- Mixed cutaneous and systemic patterns of NSAID sensitivity occur and are said to make up about 30 % of NSAID-sensitive patients. Patients show a blend of respiratory and cutaneous symptoms.
- Delayed, that is type IV, T cell-mediated reactions are seen. Some NSAID-induced delayed reactions occur as contact dermatitis, fixed drug eruption, DRESS, acute generalized exanthematous pustulosis, SJS, TEN, and nephritis.
- IgE antibodies to aspirin occur and specific assays for their measurement have been developed.
- An association of AIA with HLA-DPB1*0301 has been demonstrated in Polish and Korean populations. Other positive associations between aspirin-induced urticaria/angioedema and HLA alleles have been demonstrated or suggested.

Further Reading

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Contrast Media

10

Abstract

Reactions to iodinated contrast media range from mild inconvenience to life-threatening emergency. Histamine release can account for many of the symptoms and nonionic agents are tolerated better than ionics. Reactions can be immediate (IR) or delayed (DR). Incidences of the former are 3–4 % (ionics), 0.2–0.7 % (non-ionics), severe reactions 0.1–0.4 % (ionics), and 0.02–0.04 % (non-ionics). Up to 80 % of reactions can be avoided by using nonionic agents. For DRs, there is no difference between the incidences of reactions to each of the agents. Risk factors for IRs are a previous reaction to a contrast medium, bronchial asthma, cardiac disease, and highly allergic subjects; for DRs, a previous reaction, use of β-blockers, treatment with IL-2, history of drug allergy, and contact allergy. Diagnosis of IRs is based largely on skin tests; IgE antibodies have not been convincingly demonstrated. Breakthrough reactions have occurred following corticosteroid and H_1 antagonist premedication. Gadolinium-based agents, especially the linear chelates, have been associated with nephrogenic systemic fibrosis. They show an adverse reaction incidence of about 0.48 % and 0.01 % for anaphylaxis. Overall, given the large number of contrast media administered, they are one of the safest drugs.

Along with the development and introduction of powerful investigative techniques and advanced diagnostic equipment, contrast media have helped place clinical diagnostic radiology irreplaceably at the center of diagnostic medicine today. By increasing the contrast of anatomical structures that are otherwise not easily seen and discriminated, contrast media allow the visualization of details of internal tissues such as blood vessels, intestine and the various organs. The agents are now so widely and frequently used that they are said to be the most commonly used drugs in the history of modern medicine. Contrast media are not dyes. For contrast media-aided visualization of the body's internal structures, X-ray imaging techniques are most often used. Information was originally recorded on X-ray film, but that has now been largely superceded by digitized images using computer-based methods of recording, storage, and display.

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_10, © Springer Science+Business Media, LLC 2013

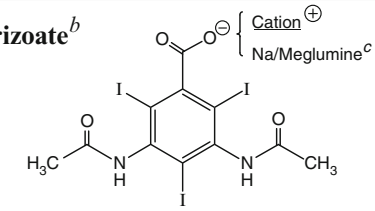
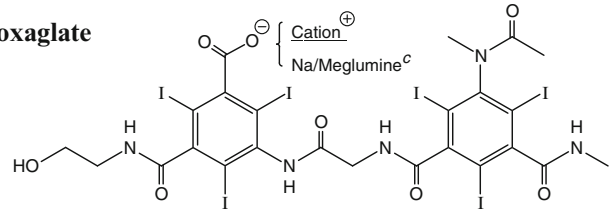
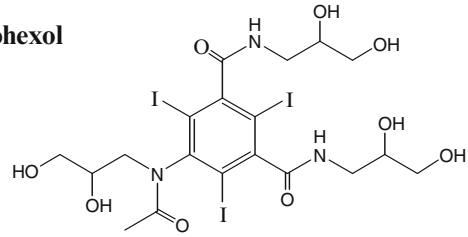
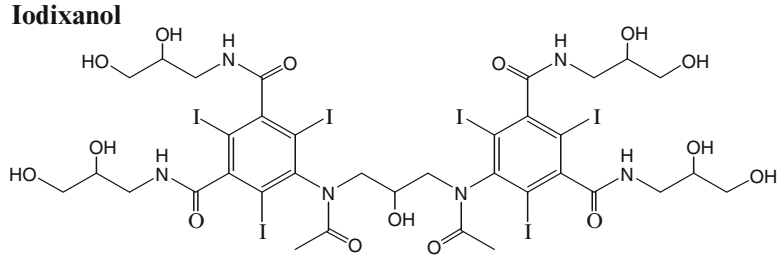
Instead of passing a single X-ray beam through the body, a computerized tomography scanner takes X-ray images at many different angles. A computer is used to work out the relative density of the emerging X-rays and ultimately a 3D image can be constructed. Sometimes structures cannot be visualized by X-rays alone, even with the aid of contrast media, for example, the cord of nerve roots. Here, specific contrast media can be employed and visualized directly using magnetic resonance imaging (MRI).

In an X-ray examination of a patient some of the X-rays are scattered in all directions and some are absorbed by the different tissues: this is known as attenuation of the rays. The amount of X-rays absorbed depends on the thickness and density of the material (for example, gas in the lungs verse lung tissue) in the path of the rays and, importantly, its chemical composition. Since the absorption of X-rays increases with the number of electrons, the chemical composition of a tissue can be thought of as the average or effective atomic numbers of all the atoms involved. This is so since the atomic number (Z) of an atom is the number of protons in the nucleus of an atom and, in an electrically neutral atom, Z is equal to the number of electrons in the atom. In addition to the contribution of density, being able to distinguish, for example, soft tissue from bone on a radiograph, is a consequence of the low average atomic number of the soft tissue contrasted with the significantly higher average atomic number of the calcium-containing bone. Effective atomic numbers and densities (expressed as g/cm^3) are 7.42 and 1 for water, 7.46 and 1 for muscle, 5.92 and 0.91 for fat, and 20 and 1.55 for calcium. If two tissues have similar densities, thickness, and average atomic numbers, visualizing and distinguishing the tissues are more difficult and may not be possible. Although this situation occurs commonly in diagnostic radiology, visualization and contrast can be artificially altered by increasing the average atomic number of a structure. This is often achieved by administering a liquid of high average atomic number, for example, to the blood to visualize blood vessels.

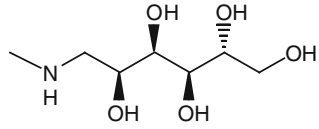
10.1 Iodinated Contrast Media

Contrast media are substances that affect the attenuation of X-rays, thus changing the contrast seen in X-ray images. Introduction of gases, as in the examination of the gastrointestinal tract, is an example of the application of negative contrast media where there is a reduction in the attenuation of X-rays, but most contrast media are positive or radio-opaque, that is, they increase the attenuation of X-rays. Since X-ray absorption increases with the number of electrons (that is the atomic number), the presence of atoms of high atomic number will absorb more X-rays than atoms of low atomic number such as hydrogen ($Z=1$), carbon (6), nitrogen (7), and oxygen (8). In one of the most successful examples of improvement in clinical diagnostics, the sciences of pharmacology and synthetic medicinal chemistry cooperated to increase the water solubility and attenuation of contrast media while at the same time reducing toxicity. This was achieved by the introduction of iodinated compounds in an evolving program of increasing effectiveness. The use of iodine in a water-soluble form for contrast imaging began in the 1920s with sodium iodide and then monoiodinated pyridine derivatives, but toxicity and poor contrast results led on to the second-generation di-iodinated pyridines. The first big breakthrough came in the 1950s with the lower toxicity but still very hyperosmolar sodium and meglumine (an amino sugar derived from sorbitol) salts of triiodinated benzoic acid. Since, for better tolerance, the osmolalities of the injected contrast medium and body fluids should be as close as possible, another significant advance was the introduction in the 1970s of nonionic iodinated contrast media. By converting the carboxyl group of triiodobenzoic acid to the amide, dissociation in solution could no longer occur and the iodine:ions/particle ratio was changed from 1.5:1 (three iodine atoms:two ions) for high-osmolality contrast media for, example, diatrizoate, to 3:1, a contrast medium of lower osmolality, for example, iohexol (Tables 10.1 and 10.2). In addition to the low-osmolar nonionic

Table 10.1 Examples of structures from each of the four different categories of iodinated contrast media[a]

Category	Drug name and structure
Ionic monomer Acetrizoate Diatrizoate Iodamide Ioglicate Iothalmate Metrizoate	**Diatrizoate**[b] 
Ionic dimer Ioxaglate	**Ioxaglate** 
Nonionic monomer Iobitridol Iohexol Iomeprol Iopamidol Iopentol Iopromide Ioversol	**Iohexol** 
Nonionic dimer Iodixanol Iotrolan[d]	**Iodixanol** 

[a]All for angiography or urography unless otherwise stated
[b]Also called amidotrizoate
[c]Meglumine:



[d]A myelographic contrast medium

compounds, low osmolality was also achieved by preparing ionic dimers such as ioxaglate (Tables 10.1 and 10.2) where only one of the carboxyls of the two linked triiodinated aromatic rings was converted to an amide, thus producing two ions in solution with a total of six iodine atoms and a ratio of 3:1. The osmolality of this ionic dimer is a little less than the osmolality of, for example, the nonionic monomer iohexol, but both are still twice as osmolar as human blood. Contrast media with approximately the same osmolality of blood were finally produced in the 1980s with the introduction of nonionic dimers containing six iodine atoms for each non-dissociating molecule, for example, iodixanol (Tables 10.1 and 10.2).

Table 10.2 Evolution of the development of contrast media with fewer side effects and low toxicity. Comparison of some important properties influencing the effectiveness and toxicity of drugs in the different categories of iodinated contrast media

Category	Drug example[a]	Iodine content (mg/ml)	Ratio iodine atoms to number of ions	Osmolality (mOsmol/kg H_2O)[b]	Number of times[c] more osmolar than blood[b,d]	Viscosity (cP_s at 37 °C)
Ionic monomer	Diatrizoate[e,f]	306	3:2 or 1.5:1	1,530	5	5.0
Ionic dimer	Ioxaglate[f]	320	6:2 or 3:1	580	2	7.5
Nonionic monomer	Iohexol	300	3:1	640	2	6.3
Nonionic dimer	Iodixanol	320	6:1	290	Iso-osmolar[b]	11.4

[a]See structures Table 10.1
[b]Different contrast media in the same group may show different osmolalities, e.g., iotrolan osmolality = 320 mOsmol/kg H_2O and is more osmolar than blood (290 mOsmol/kg H_2O)
[c]Approximately
[d]At 300 mg iodine/ml
[e]Also known as amidotrizoate
[f]Used as the sodium and/or meglumine salts

The iodine atom has an atomic radius of 140 pm (1.4 Å) (empirically measured) which is significantly larger than the atomic radii from, for example, carbon (70 pm), hydrogen (25 pm), oxygen (60 pm), nitrogen (65 pm), sulfur (100 pm), and chlorine (100 pm). To obtain a visual image of how the presence of iodine atoms dominates the volume in space of iodinated contrast media molecules, the two-dimensional structures of the four contrast agents, containing either three or six iodine atoms and shown in Table 10.1, are represented three-dimensionally as CPK models in Fig. 10.1. The presence of the bulky iodine atoms significantly influences the physical and chemical properties of the contrast media and produces structures that, as potential immunogens (antigens and allergens), are relatively unique.

10.2 Usage and Safety of Contrast Media

Iodinated contrast media are one of the most often administered and safest pharmaceutical products used today not only in radiology but in all areas of medicine. In 2005 it was estimated that worldwide administrations of contrast media exceeded 75 million per year and it is safe to assume that this figure grows annually. Unlike most drugs, they are not designed to have a specific therapeutic action; in fact, the more pharmacologically inert they are the better. With normal renal function, the iodinated agents are cleared almost completely by the kidneys with a half-life of only 1–2 h. Although most often used intravenously, contrast media can be given into arteries, the abdomen, and intrathecally, and numerous surveys in hospitals throughout the Western world attest to their heavy and widespread usage. As diagnostic technologies increase in effectiveness and sophistication, new agents and procedures become accepted as standard practice, and costs become more affordable, contrast media usage will inevitably increase. A trend that will almost certainly continue is the preference for the better-tolerated nonionic compounds and this will be driven by demand from both doctor and patient. For example, even in the late 1990s, surveys revealed that this preference was already clear in many areas of the USA. In one report, 43 % of hospitals surveyed in the southeast of the country used nonionic contrast media 100 % of the time while 71 % used them more than 75 % of the time. To reduce the usage of the nonionic agents, hospitals often introduced selective protocols.

As with every administered pharmaceutical agent, adverse reactions to iodinated contrast media do occur, but the incidence is low. Reactions can be unrelated to the dose or concentration of the solution administered (see below) or they can be dose dependent. An important contributor to the latter category is the osmolality of the administered agent which is responsible for the feelings of discomfort,

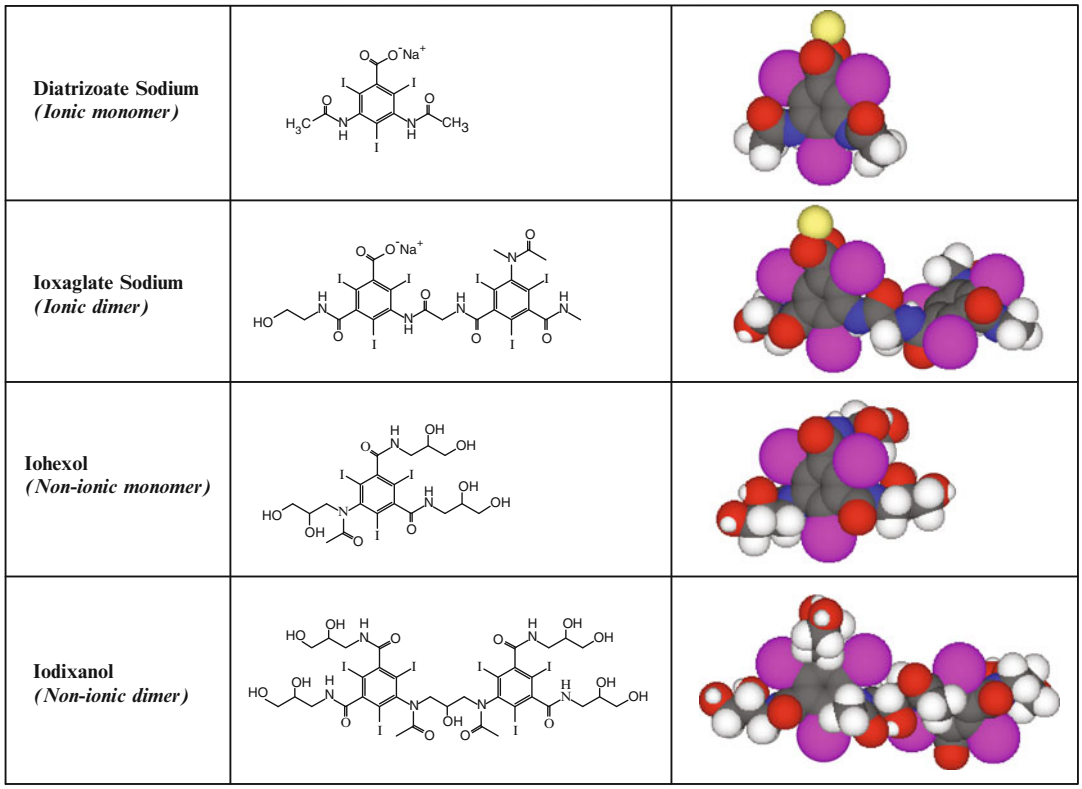


Fig. 10.1 2D structures and CPK models of some commonly used ionic and nonionic iodinated contrast media. Note the bulk of the iodine atoms relative to the rest of the molecules

heat, and pain and which may also provoke disturbance of the electrolyte balance in small children, renal problems, and damage to the blood–brain barrier. As already indicated, the tolerance of contrast media increases as the osmolality approaches the osmolality of serum. Also related to the concentration used are the viscosity, the hydrophilicity/lipophilicity balance, protein-binding capacity, and histamine-releasing properties of the contrast medium. Viscosity increases with molecular weight so the viscosities of the dimer solutions are higher than the solutions of monomers. The practical implications of a more viscous solution are the greater force required for injection, especially through thin catheters, and slow flow influencing the visualization of tissues. Some additional adjustments made to reduce toxicity include adjusting the pH to neutral, adding calcium ions to reduce cardiac toxicity, and altering the number of hydroxyl groups to decrease neural toxicity.

10.3 Adverse Reactions

10.3.1 Classification and Symptoms

10.3.1.1 Acute (Immediate) Reactions

Iodinated contrast media used today have been carefully developed with the aim of maximizing their effectiveness for tissue visualization while at the same time minimizing toxic effects. With the relatively safe agents used today, of more concern than dose-related intolerance and toxicity outlined above are adverse reactions covering a range of severities that are mostly independent of dose or concentration. Severity is, in fact, a convenient and useful way of categorizing these reactions since this approach is clinically relevant and provides a guide for subsequent treatment. As with the so-called toxic reactions, the adverse reactions are small in number relative to the millions

Table 10.3 Adverse reactions to contrast media. Some of the main symptoms seen in acute and late reactions

Acute (immediate) reactions[a]			Late (delayed) reactions[b]	
Mild	Moderate	Severe	Reactions to: iodinated contrast[c,d] media	Reactions to: gadolinium[e] contrast media
Nausea, mild vomiting Urticaria Itching	Severe vomiting Marked urticaria Bronchospasm Facial/laryngeal edema Vasovagal attack	Hypotensive shock Respiratory arrest Cardiac arrest Convulsions	Nausea, vomiting Headache Musculoskeletal pain Fever Skin reaction[g]	Nephrogenic systemic fibrosis[f]

Data adapted from ESUR Guidelines of Contrast Media. European Society of Urogenital Radiology, Version 7.0. View at http://www.esur.org/ESUR-Guidelines.6.0.html

[a]Reactions occur within 1 h of injection of contrast media

[b]Reactions occur 1 h to 1 week after injection of contrast media

[c]Many symptoms described not related to contrast media

[d]Risk factors: previous contrast media reaction; interleukin-2 treatment. Prophylaxis generally not recommended Patients who had a previous serious late reaction can be given steroids as premedication (see Table 10.4)

[e]Reactions to gadolinium contrast media of lower risk than with an iodinated contrast media

[f]Nephrogenic systemic fibrosis usually presents after 1 week but may occur earlier (see Sect. 10.7.2)

[g]Usually mild to moderate and self-limiting. Management is similar to other drug-induced skin reactions

of doses administered each year. A number of professional bodies including, for example, The American College of Radiology and the European Society of Urogenital Radiology, have issued classifications and guidelines based on a primary division of acute (or immediate) and late reactions with the former division subdivided further into mild, moderate, or severe reactions. Acute reactions are those that occur within about 1 h of the administration of contrast media. The cutoff time of 1 h for a reaction to be classified as acute is, of course, somewhat arbitrary and it is a figure disputed by some who argue that findings with some patients show that the cutoff point should be extended to 2 or even 3 h. A similar problem of deciding the time when the designations "immediate" ends and "delayed" begins is seen in other drug allergies. The question is probably best resolved by the patient's symptomatology.

Signs and symptoms commonly listed for the three different acute reaction categories include mild reactions (generally self-limiting without evidence of progression)—nausea, vomiting, cough, headache, itching, pallor, flushing, and chills; moderate reactions (require treatment but not immediately life-threatening)—tachycardia/bradycardia, bronchospasm, laryngeal edema, marked urticaria; and severe reactions (life-threatening)—hypotensive shock, cardiac and respiratory arrest, severe laryngeal edema, and convulsions. Table 10.3 sets out the classification of acute non-renal adverse reactions to contrast media and the associated symptoms listed by the European Society of Urogenital Radiology in their ESUR Guidelines on Contrast Media.

10.3.1.2 Late Reactions

Late reactions become apparent more than 1 h and up to about 1 week after contrast media exposure. Excluding contrast media-induced nephropathy, the symptoms most commonly seen include nausea, vomiting, headache, and cutaneous reactions (Table 10.3) which tend to be self-limiting and include maculopapular rash in over 50 % of affected patients, xanthema, urticaria, and usually pruritus. In rare cases, cutaneous reactions may progress to a cutaneous vasculitis or even a Stevens–Johnson-like syndrome. Late reactions may often be missed since patients generally leave the department sooner than an hour after administration of the contrast preparation, and because the delayed reactions are so often self-limiting, the radiologist may remain unaware of them. Recent reports, however, of delayed skin reactions and a

few cases of serious delayed reactions involving hypotension, shock, and angioedema following intravascular injection of nonionic iodinated dimers highlight the potential dangers of late reactions (see below) occurring in the absence of direct medical awareness and supervision. Gadolinium-based contrast media are referred to in Table 10.3 for the sake of completeness. These agents are discussed later in this chapter.

10.3.2 Incidence of Reactions

10.3.2.1 Acute Reactions

The largest study so far of the incidences of adverse reactions to different contrast media was reported by the Japanese Committee on the Safety of Contrast Media in 1990. In this prospective study of 337,647 cases, 169,284 cases (50.1 %) received ionic contrast media and 168,363 (49.9 %) received nonionic contrast media. Adverse drug reactions occurred in 12.66 % of the ionic contrast media group and 3.13 % of the nonionic group. For severe adverse reactions the corresponding figures were 0.22 % and 0.04 %, respectively, with one death occurred in each group. The authors of the study concluded that "non-ionic contrast media significantly reduce the frequency of severe and potentially life-threatening adverse drug reactions to contrast media at all levels of risk and that use of these media represents the most effective means of increasing the safety of contrast media examinations." While the incidence of reactions to high-osmolar ionic contrast media in the Japanese survey is higher than most estimates, it is clear that reactions to these agents occur within the range of about 2–8 % with a figure of around 3 or 4 % perhaps being most likely. The estimated reaction frequency for the low-osmolar nonionic agents ranges up to a maximum of about 3 %, but figures of 0.2–0.7 % have been deduced in a number of studies. Figures for the incidences of severe reactions are much more settled being 0.1–0.4 % for ionic and 0.02–0.04 % for nonionic agents. For reactions judged to be severe, the corresponding figures are 0.04 % and 0.004 %. Fatal reactions occur rarely and do not differ between the low- and high-osmolality agents. The mortality rate has been estimated to be in the range 1 in 100,000 to 1 in 170,000.

In summary then, it can be said that as well as provoking a higher incidence of adverse reactions, high-osmolar ionic contrast media cause reactions that are more severe than the low-osmolar nonionic contrast media-induced reactions and the nonionic preparations are less distressing for the patient overall. Although severe reactions with high-osmolar ionic contrast media are still rare, they are more frequent than severe reactions to low-osmolar nonionic media. Up to 80 % of the reactions to the ionic agents can be avoided by substituting a nonionic medium.

10.3.2.2 Late Reactions

Obtaining reliable and relevant information on the frequency of late reactions to contrast media is not easy for a number of the usual reasons related to data collection but particularly because of the relatively larger time interval between the injection of the agent and the appearance of symptoms. And, of course, the bigger the time interval, the more difficult it is to be sure that the symptoms were caused by the contrast medium. Most studies show that there is no significant difference in the incidences of late reactions between ionic and nonionic media or between the different nonionic preparations. Although figures as low as 0.52 % and as high as 23 % have been reported, the incidence of reactions in the first 24 h appears to be about 4 % settling to about 1–3 % over a 7-day period. The nonionic compound iopamidol showed an incidence of 5.5 % of late skin rashes in a survey of 1,381 patients.

A curious seasonal variation in the occurrence of late adverse skin reactions has been reported from Finland. In a study of a possible relationship between sun exposure and late reactions in 4,875 adults who had received an iodinated contrast medium, a 3-month (April to June) peak in the incidence of reactions was seen. This period included 35 % of all events and most of the reactions occurred on sun-exposed areas of the body, leading the authors to conclude that a possible explanation for the observations was the photosensitizing effect of the contrast media.

Table 10.4 Risk factors for acute reactions to iodinated contrast media and procedures and strategies to reduce the risks

Risk factors	
Patient related	Patient with history of – Previous reaction to iodinated contrast media – Asthma – Allergy requiring medical treatment
Contrast media related	High-osmolality ionic contrast media
To reduce the risk	
For all patients	Use a nonionic contrast medium Keep patient in Radiology Dept. for 30 min after injection of contrast media Have drugs and equipment for resuscitation readily available (see Table 10.5)
For patients at increased risk of reaction	Consider alternative test, i.e., not requiring a contrast medium Use different iodinated contrast media for previous reactors Consider premedication[a]

Data adapted from ESUR Guidelines on Contrast Media. European Society of Urogenital Radiology, Version 7.0. View at http://www.esur.org/ESUR-Guidelines.6.0.html

[a]Suitable regime: prednisolone 30 mg (or methylprednisolone 32 mg) orally given 12–2 h before contrast media (see discussion in Sect. 10.6)

10.3.3 Risk Factors

10.3.3.1 Acute Reactions

Risk factors for acute reactions to contrast media are summarized in Table 10.4. The most significant risk is for patients who have experienced a previous immediate reaction to an iodinated contrast medium. Reexposure to the same or structurally similar ionic preparation is said to carry with it a 21–60 % risk of a repeat reaction. This risk is one-tenth as great if a nonionic contrast medium is substituted for the repeat injection. Comparable figures for nonionic media used for the initial and the repeat administrations do not seem to be available. Other important risks are bronchial asthma, the use of β-blockers, cardiac disease, and subjects who are highly allergic. Procedures and efforts to reduce the risks of an acute reaction are set out in Table 10.4. Some of the points, for example, use of nonionic media, substituting a different contrast medium, and keeping patients under surveillance longer, have been considered above. Physicians using contrast media should be trained to recognize, test for, and treat anaphylaxis. Drugs and instruments that should be close at hand for acute reaction emergencies following administration of contrast media are listed in Table 10.5.

Table 10.5 Drugs and instruments that should be in the examination room following injection of contrast media

Oxygen
Epinephrine (adrenaline) 1–1,000
Histamine H_1-receptor antagonist in injection form
Atropine
Beta2-agonist in metered dose inhaler form
IV fluids—physiological saline or Ringer's solution
Anti-convulsive drugs (eg., diazepam)
Sphygmomanometer
One-way mouth breather apparatus

Information from ESUR Guidelines on Contrast Media. European Society of Urogenital Radiology, Version 7.0. View at http://www.esur.org/ESUR-Guidelines.6.0.html

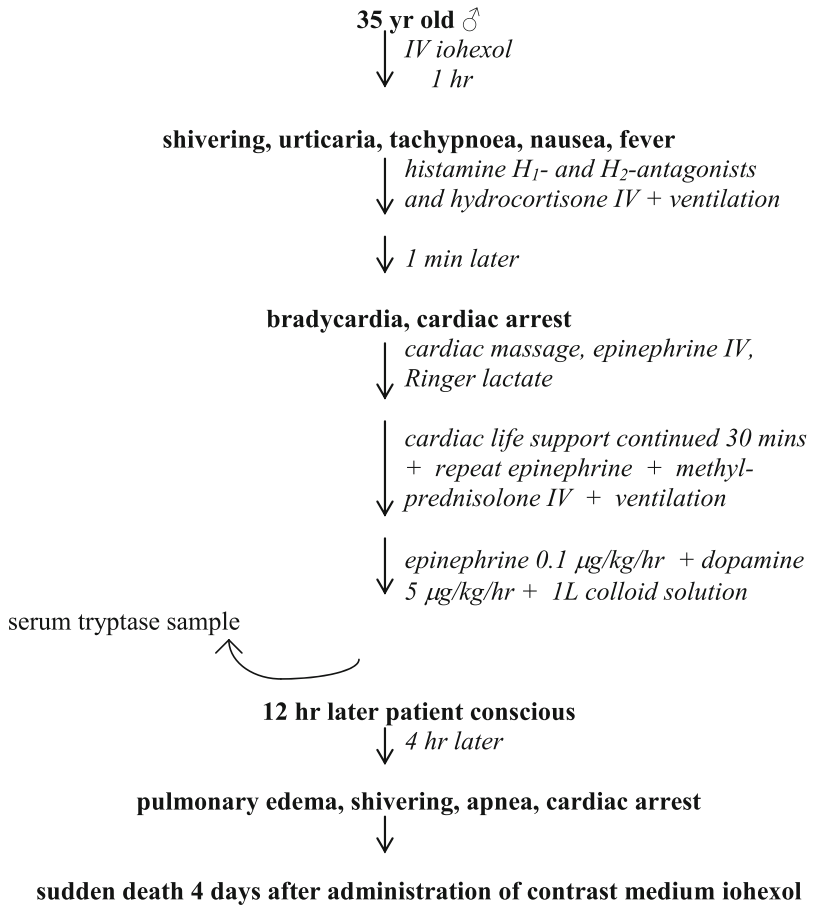
10.3.3.2 Late Reactions

Risk factors for a late skin reaction following administration of an iodinated contrast medium include current and up to 2 years past treatment with interleukin-2 (IL-2), a history of drug allergy or contact hypersensitivity, and a history of reaction to a previous contrast medium. Late reactions are more common in patients who reacted previously, especially if the same contrast medium is administered (see below). The latter fact is interesting since it suggests that the mechanism of the late reaction with its demonstration of memory may be immunologically and, in particular, T cell mediated.

10.3.4 Biphasic Reactions

About 20 % of adverse reactions to iodinated contrast media are biphasic in nature and although severe biphasic reactions are rare they are of concern since they can be life-threatening. The second

Table 10.6 Fatal biphasic reaction following anaphylaxis to a nonionic contrast medium



Data from Choudhury M et al. Indian J Anaesth. 2011;55:631

or late phase usually occurs after an asymptomatic period of from about 1 h up to 3 days or more (see Sect. 10.5.1) and it can be less, equal, or more severe than the immediate reaction. A recently reported case summarized in Table 10.6 dramatically illustrates why, after an anaphylactic reaction to a contrast medium, a physician should be wary of a second-phase response and patients should be made aware of the risk on discharge. Although there appears to be no clinical features that can indicate the possibility of a biphasic acute reaction, it seems that patients who experience the delayed response require higher doses of epinephrine to control their initial reaction.

10.4 Mechanisms of Adverse Reactions to Iodinated Contrast Media

The range and diversity of adverse effects provoked by contrast media remain poorly understood and hence difficult to categorize. For the allergist and clinical immunologist used to thinking of immediate reactions as type I allergic responses mediated by IgE antibodies and delayed reactions as type IV hypersensitivity reactions mediated by antigen-specific effector T cells, adverse reactions to contrast media, divided

as they often are into acute and late reactions, do not fit neatly into the conventional mechanism-based classification (refer to chapter 2).

10.4.1 Anaphylactoid and Anaphylactic Reactions

The serious severe reactions induced by contrast media show close similarity to other drug-induced anaphylactoid and anaphylactic reactions discussed at length in earlier chapters, but, as with drugs such as neuromuscular blocking agents, the opioids and other histamine-releasing agents (Chaps. 7 and 8), distinguishing the true, immunologically based anaphylactic reactions from pseudoallergic or anaphylactoid reactions caused by release of inflammatory mediators is generally difficult.

10.4.1.1 Histamine Release

Preformed histamine when newly and rapidly released by degranulation of mast cells and basophils accounts for most of the primary manifestations seen in an anaphylactic reaction (Sect. 3.2.5.1), but the usefulness of assessing histamine release in vitro has not led to general application of the strategy for the diagnosis of drug allergies (see Sect. 4.5.2). Contrast media of high-, low-, and iso-osmolality are well-known releasers of histamine, a property demonstrated in many studies over a period of more than 40 years, so it is not surprising that the experimental findings have led to the suggestion that released histamine may be the mechanism of severe anaphylactic-like reactions to these agents. High- and low-osmolality contrast media have been shown to produce a rise in plasma histamine that peaks and falls back to baseline over a period of about 10 min, but there seems to be no direct relationship between the magnitude of the rise and the severity of the reaction. Even so, a relationship to moderately severe symptoms such as vomiting and rash was suggested and this raised the question of the probability of histamine's contribution to the more severe reactions to contrast media. The fact that high-osmolar contrast media are responsible for more severe acute reactions than the low-osmolar compounds seems to fit with research findings showing that the high-osmolar compounds release more histamine than the low- and iso-osmolar contrast media, although some studies have concluded that hypertonicity is not an absolute requirement for contrast media-induced histamine release from human basophils in vitro. Another significant conclusion from in vitro experiments was the finding that bloods from previous reactors release a larger percentage of histamine than bloods from nonreactors. Just as the opioid drugs show differences in the amount of histamine they release and in the sites where release occurs (Sect. 8.4), contrast media show similar anatomical selectivity releasing histamine and tryptase from human lung and heart mast cells but not from skin mast cells. Depending on the particular contrast medium injected, this selectivity may influence the symptoms and severity of any subsequent reaction to the drug. Among the ionic agents, meglumine salts are more potent releasers of histamine than the corresponding sodium salts.

Of course, with any case of drug-induced histamine release, the key question to consider is the mechanism underlying the release. Release might proceed in an immediate reaction by direct action of the drug on the mast cells and/or basophils or it might be immunologically mediated as in anaphylaxis mediated by drug-reactive IgE antibodies. However, no cell membrane receptors for any iodinated contrast medium have been identified so far and proving an immunological basis even for the most serious acute reactions has been difficult. For the large majority of patients with contrast medium-induced symptoms appearing within 1 h, IgE antibodies complementary to the culprit drug cannot be demonstrated (see below for a further discussion of IgE antibodies and contrast media).

Activation of complement by contrast media to produce the anaphylatoxins $C3_a$ and $C5_a$ has also been proposed as the mechanism for contrast media-induced histamine release. These pro-inflammatory complement fragments act via specific receptors on endothelial and mast cells and can induce a shock-like reaction similar to that seen in type I allergic responses. As yet, however,

with apparently only a single study showing no differences in anaphylatoxin levels between patients and controls, there appears to be no compelling evidence either way to accept or refute this proposal.

There are many obvious problems to confront in any study designed to examine the role of histamine (and other inflammatory mediators) in adverse reactions to contrast media. Plasma histamine levels peak within 1–8 min following direct treatment with most histamine-liberating drugs (see Sect. 8.4.3) and within 5–15 min after antigen challenge, returning to baseline about 30 min and 60 min later, respectively. There are obvious difficulties in being able to select and study the right patients at the right time and within the required short time frame. Obtaining results, even from small numbers of patients experiencing a severe immediate reaction, involves many difficulties plus an element of luck on the investigator's part. The rarity of severe reactions emphasizes the paucity of suitable subjects available for contrast media-induced histamine release studies and probably results in the examination of too many patients undergoing minor reactions. Ideally, one would like to be able to perform specific skin tests, specific IgE antibody assays (both with the appropriate controls which include skin testing normal subjects with contrast media and IgE-contrast media inhibition studies), tryptase sampling at suitable times, and quantitation of released histamine.

10.4.1.2 The Question of the Involvement of Contrast Media-Reactive IgE Antibodies

In true type I immediate allergic responses to drugs, just as with immediate reactions to common inhalant, food, and venom allergens, IgE antibodies mediate the reactions and one would therefore anticipate the presence of contrast media-reactive IgE antibodies in the sera of subjects showing immediate reactions, especially severe ones, following injection of contrast media.

Intriguingly, however, positive tests for serum antibodies have been extremely rare and when found they have been in patients with severe acute reactions (see Sect. 10.5.2 below). The subject has been bedeviled by the inconsistencies of results obtained from investigations of fatal reactions and from a broad group of mild to severe reactors suffering an acute (immediate) adverse reaction. There are also the questions of who is to be investigated and when investigations should be pursued. Despite the fact that many drug reactions occur on first exposure to the drug (for example with neuromuscular blockers, quinolones, and a wide range of different drugs in some individuals), a belief persists that without prior sensitization to the drug no immunological response, and in particular an IgE antibody response, can occur. Much the same attitude can sometimes be found toward breakthrough reactions to contrast media after premedication with antihistamine and cortisone. The problem with these poorly informed approaches is that some patients with genuine acute reactions that may be antibody mediated, and even potentially anaphylactic, remain unstudied and undetected.

The question of whether or not iodinated contrast media elicit antibody formation, and in particular IgE antibodies, has been considered from the viewpoint of whether the drugs *can* stimulate antibody formation in the first place. Using what was described as "highly favorable conditions for the production of antibodies" that included *Nippostrongylus brasiliensis*-infected Hooded Lister rats that are said to be better at producing antibodies than other strains and the proven adjuvant *Bordetella pertussis*, meglumine ioglycamate and sodium/meglumine diatrizoate conjugated to carrier proteins were employed as immunogens and any subsequent homocytotropic antibody formation was monitored by the passive cutaneous anaphylaxis (PCA) technique. No evidence for the formation of reaginic antibodies was found leading to the conclusion that "it therefore seems unlikely that the majority of adverse reactions to radiographic contrast media are allergic in nature". Apart from the obvious doubts about extrapolating results in rodents to humans, a number of different drugs in their original unbound state may allergically sensitize and elicit antibody responses by direct interaction with immune cells and, as with the neuromuscular blocking drugs for example, the sensitizing agent

may not be the drug in question. Perhaps another study that should be considered is the injection of a large number of rats or mice with contrast media in an attempt to mimic the situation with human patients and then look for reaginic antibody responses. Considering the human situation, one might expect that for this experiment to be informative, a large number of animals would have to be examined. A clue to what might be a possible immunological function of contrast media in vivo is the finding that iopamidol had a marked adjuvant effect on the production of antihapten IgE and IgG1 antibodies in mice and enhancement of antibody production was associated with IL-4 release. Antibodies to the contrast medium were not detected.

In the absence of easy-to-perform and reliable assays for the detection of contrast media-reactive IgE antibodies in patients' sera, some other less direct but arguably more biologically and clinically relevant test procedures have occasionally been employed. With both the basophil activation and Prausnitz–Kustner (P–K) tests, the interaction between allergen (contrast medium) and IgE antibodies takes place at the basophil (or mast cell) surface, thus mimicking the in vivo situation. However, for different reasons, neither test has become, nor is likely to soon become, a routinely and widely applied procedure for the diagnosis of adverse reactions to contrast media or any other drug (see below).

An interesting finding with the low-osmolar dimer ioxaglate may have some relevance to the question of whether or not contrast media-reactive IgE antibodies are part of the mechanism underlying some reactions to contrast media. Some (but not all) study comparisons have reported more reactions to the dimers than to higher osmolar ionic media, a similar incidence of severe reactions by the two, and a lower incidence of monomer-induced fatal reactions. This has prompted the speculation that the dimers may be antigenically divalent, thus allowing them to bridge adjacent antibody combining sites of mast cell-bound IgE molecules (see Sect. 3.1.2). The prediction is that if this is so, dimeric iodinated contrast media might be more likely to induce mediator release and anaphylaxis than univalent monomers. A similar prediction was advanced for the neuromuscular blocking drugs (Sect. 7.4.2.3).

10.4.1.3 Activation of the Kinin System and Bradykinin

See Sect. 3.2.8.5.2 and Fig. 3.13) for a summary of the kallikrein–kinin system.

Bradykinin, a potent vasoactive nonapeptide, is formed by interaction of factor XII (Hageman factor), high molecular weight kininogens and prekallikrein on negatively charged surfaces (for example, silicates), on macromolecular surfaces such as collagen of connective tissue, heparin and mucopolysaccharides, and on the surfaces of cells together with some specialized proteins including complement component $C1_q$. Bradykinin is also produced by a mechanism bypassing factor XII that involves protease activation of prekallikrein. Cell activation during inflammation and heparin release is thought to activate the plasma cascade leading to bradykinin release and, via its inflammatory and hypotensive effects and capacity to induce tissue hyperresponsiveness, its detrimental role in asthma, anaphylaxis, and other allergic conditions. Bradykinin is degraded by carboxypeptidase N and angiotensin-converting enzyme and it has been claimed that the latter enzyme is inhibited in asthmatics with active bronchospasm and by ionic contrast media at concentrations attainable in the circulation. Such an action reducing or preventing the hydrolysis of bradykinin and therefore limiting its effects might help to explain the increased susceptibility of asthmatics to contrast media. More findings advanced to support the role of the kinin system in elucidating the mechanism(s) of contrast media-induced adverse effects are the reported increases in the plasma of negatively charged heparin-like contact activators and so-called cryptic soluble negatively charged surfaces in subjects who react to contrast media and in asthmatics. An indirect action of bradykinin also contributes to its pro-inflammatory effects. By activating phospholipase A_2, the peptide stimulates the release of arachidonic acid from phospholipids leading to the production of prostaglandins and leukotrienes via the cyclooxygenase (Sect. 9.4.1) and lipoxygenase (Sect. 3.2.5.2.1) pathways. Currently it is difficult to

judge the importance of these proposed mechanisms to explain contrast media reactivity since other supporting and follow-up evidence has not been forthcoming.

10.4.2 Delayed Reactions

Delayed-type hypersensitivity reactions to iodinated contrast media are said to be rare, but there are some claims that 1–3 % of patients injected intravenously with the agents experience such reactions. Symptoms include persistent pain at the injection site, nausea, vomiting, flu-like symptoms, angioedema, dyspnea, fixed drug eruption, and maculopapular exanthema. Only a small number of cases showing documented evidence supporting a diagnosis of delayed hypersensitivity reactions with positive delayed skin tests have been reported. Maculopapular exanthema is the most commonly seen reaction, accounting for over 50 % of patients with a delayed reaction to a contrast medium. In one well-documented case, a 61-year-old patient with no history of allergy or prior exposure to contrast media developed generalized maculopapular exanthema 7 days after injection of the nonionic agent iopamidol. Three months after the reaction, iopamidol was again administered. Despite premedication with prednisone and cetirizine commencing 3 days before injection, the patient reacted 1 day later with generalized, confluent macular exanthema accompanied by severe itching and enanthema of the oral mucosa. Patch testing showed a positive allergic reaction to iopamidol. In follow-up patch tests, iohexol and ioversol as well as iopamidol gave positive reactions, but the ionic agent sodium amidotrizoate proved negative. Although the ionic compound shares a 2,4,6-triiodobenzene core nucleus with the three nonionic drugs, it is structurally different in the groups attached at positions 1 and 3 of the ring where the latter compounds each have acetamido groups. This structural difference accounts for cross-reactivity of the nonionic agents and the absence of it for the ionic drug.

Some investigators believe that cell-mediated hypersensitivities are responsible for most of the non-immediate reactions to iodinated contrast media but a number of different cellular and cytokine-driven processes may be involved, making this a difficult and complex problem to study. Positive patch and delayed intradermal tests, the presence of T cells at skin test sites, positive responses to provocation testing, immunohistological findings, and contrast media-induced proliferation of T cells from patients with delayed reac tions to the agents all give weight to the belief that these late reactions are mediated by T cells. Results from a recent investigation of possible pathways for recognition of iodinated contrast media by T cells suggested that two mechanisms of T cell stimulation were operative. Contrast media-specific T cell clones (TCC) were generated from contrast media-allergic patients and a specific T cell receptor (TCR) was transfected into a mouse T cell hybridoma. Proliferation and IL-2 and Ca^{2+} assays were performed using HLA-DR-matched or mismatched antigen-presenting cells (APC). An increase in intracellular Ca^{2+} within seconds of the addition of drug, cell proliferation, and IL-2 secretion in the presence of glutaraldehyde-fixed APCs suggested that stimulation occurred by direct binding to the major histocompatibility complex (MHC)–TCR complex. With other TCCs, abrogation of presentation by glutaraldehyde-fixed APCs, failure to wash away drug from APCs preincubated with contrast media, and an optimal pulsing time of 10–20 h suggested processing by APCs.

The precise mediators of contrast media-induced allergic reaction are also largely unknown, but cytokines are known to participate in both immediate and late reactions. Investigations so far of possible cytokine involvement following contrast media injection revealed an early increase (after 1 h) in IL-2 followed by a delayed increase of IL-4 and IL-6, indicating a Th1 to Th2 shift in late adverse reactions. The highest histamine levels were seen in late reactors 24 h after injection of the contrast medium. TNF-α did not show any significant change. T cell studies, still in their early days and so far on small numbers of patients, have generated iodinated contrast media-specific T cell clones and demonstrated cross-reactivity with some other contrast media by some of the CD4(+) clones.

Iomeprol-specific peripheral T cells for example were shown to occur with a frequency of 0.6 %.

10.5 Tests for the Diagnosis and Study of Adverse Reactions to Contrast Media

As always, a meticulously recorded and studied history is desirable, but additional clinical and laboratory tests can contribute greatly to an accurate diagnosis and help to identify the mechanism(s) of the reaction. Skin tests, detection, and quantitation of released mediators and serum IgE antibodies may all contribute to establishing a more precise diagnosis of an adverse response to an iodinated contrast medium.

10.5.1 Skin Tests

For a general discussion of skin testing, see Sect. 4.2.

Although there is no long-established and widespread diagnostic practice of skin testing patients with contrast media, results from recent studies indicate that skin testing with iodinated contrast media is a useful tool in efforts to improve the diagnosis of allergy to these agents. Some small skin test studies and tests on individuals have been carried out intermittently over the years, but a recent European multicenter prospective study carried out under the auspices of the European Network of Drug Allergy and the European Academy of Allergy and Clinical Immunology Interest Group on Drug Hypersensitivity set out, for the first time, to determine in a large study the specificity and sensitivity of skin tests in patients who experienced reactions to iodinated contrast media. Skin prick tests followed by intradermal tests and patch tests were performed on 220 patients with a reported previous hypersensitivity reaction, either immediate or delayed, to contrast media using the dilutions, procedures, and controls summarized in Table 10.7. Overall, positive skin tests were seen in 32 of 122 patients (26 %) with immediate reactions. Intradermal tests were positive in 30 patients and prick tests in only 4. An early and obvious finding was the relationship between the skin test result and the time between reaction and testing. Within 2–6 months of the adverse reaction, the percentage of positive reactors increased from the overall figure of 26 to 50 % (14/28). For patients tested at other times, that is, earlier than 2 months and later than 6 months, the figure was only 18 % (17/92).

For non-immediate reactors, a combination of intradermal and patch tests were needed to identify the maximum number of reactants. Delayed skin tests were positive in 37 of 98 patients (38 %) with only three positive in the skin prick test. Of 31 patients positive in the intradermal test, 9 delayed reactions were detected on day 1, 16 on day 2, and 6 on day 3. Patch tests required times of up to 3 days to detect all of the 22 positive patients out of 79 tested (28 %). Some patients were positive in the intradermal but not in the patch test while with some others it was the reverse. As with the immediate reactors, most reactors (29 of 62; 47 %) were detected when the tests were performed within 6 months of the reactions to contrast media; only 8 of 36 (22 %) were positive at later times. Cross-reactions between some contrast media were detected especially between those of similar structure, for example, iophexol, iomeprol, iopentol, ioversol, and the nonionic dimer iodixanol. From the wider perspective of the mystery of how some patients become allergically sensitized to some drugs (see for example, Sect. 3.1), it is interesting to note that one-third of the patients with a positive delayed skin test in this study reacted to contrast media on their first exposure to the agent. In what may turn out to be a useful observation, patients with a delayed positive skin test showed a higher number with maculopapular exanthema and a lower number with urticaria-like exanthema than the patients who were skin test negative.

The authors of the European multicenter study pointed out some limitations in their report. These included lack of provocation testing, the need for more control subjects exposed to a contrast medium but without clinical signs of a hypersensitivity reaction, the culprit drug was not

Table 10.7 Skin testing details and procedures for testing patients with suspected hypersensitivity reactions to iodinated contrast media

Test	Concentration of test solution[a] and how applied	Site of test	Reading times	Positive reaction[b,c]
Skin prick test[d]	Undiluted contrast media solution. For standard procedure, see Sect. 4.2.2	Volar forearm	After 20 min and on days 2 and 3	Immediate reaction: Wheal ≥ 3 mm after 20 min Delayed reaction: Erythematous induration at site on day 2 or 3
Intradermal test[e]	Contrast media solution diluted 1–10. Inject 0.03–0.05 ml to give 4–5 mm diam. bleb.	Volar forearm or back	After 20 min and on days 1, 2 and 3	Immediate reaction: Initial wheal increased by at least 3 mm diam. and surrounded by erythema after 20 min Delayed reaction: Erythematous induration at site at delayed reading
Patch test[f]	Undiluted contrast media Filter paper soaked in contrast media and placed in 12 mm aluminum Finn chamber Fixed on back with adhesive tape for up to 3 days	Back	Up to 3 days. Read 15 min after removal and 24 h later	For reading and scoring patch test reactions, see Sect. 4.2.4.3, Fig. 4.5 and Table 4.1

Data from Brockow K et al. Allergy. 2009;64:234
[a]All solutions were 300–320 mg iodine/ml
[b]Controls: positive—histamine solution 0.01 %; negative—saline 0.9 %
[c]Skin test sensitivity—the percentage of skin test-positive patients showing a typical hypersensitivity reaction after administration of contrast media; skin test specificity—percentage of negative controls with a positive skin test to contrast media
[d]See Sect. 4.2.2
[e]See Sect. 4.2.3
[f]See Sect. 4.2.4

always identified, a possible lack of test sensitivity, and the fact that the negative predictive value of the skin tests had not been determined. In relation to the last point, a negative predictive value of 96.6 % has recently been claimed for tests with iodinated contrast media (see Sect. 10.5.4), but, to be confident of this figure, studies with larger numbers of patients need to be done. Despite these limitations and some others, most of which are not necessarily easy to overcome, the main and most important conclusions from the first 4 years of this prospective study are that about half of the hypersensitivity reactions to iodinated contrast media have an immunological basis and skin testing, especially intradermal and patch testing, is a useful diagnostic tool that may aid the selection of a safe contrast agent for those patients who have experienced a previous reaction.

10.5.2 IgE Antibody Tests

10.5.2.1 Prausnitz–Kustner Test

This test relies on the capacity of homocytotropic antibodies or reagins to fix to human skin and be detected by subsequent injection of the suspected allergenic drug. Although a positive reaction is not immediately indicative of the involvement of IgE antibodies, their presence is often inferred if skin sensitization is prevented by prior heating of the serum to 56 °C. Prior to the realization of the possibility of the transfer of blood-borne viruses, and the introduction of easier-to-carry-out tests that specifically identify IgE, the P–K test was used in studies aimed at determining whether or not the antibody was implicated in some adverse reactions to iodinated contrast media. In one case study, a positive P–K test was taken as evidence

that an immediate reaction to ioglycamic acid was mediated by IgE antibodies.

10.5.2.2 Serum Tests

For details of the detection of drug-reactive IgE antibodies in sera, see Sect. 4.3. As already outlined, IgE antibodies to iodinated contrast media have not been convincingly and consistently demonstrated and the current consensus is that true type I IgE antibody-mediated reactions to the drugs are rare, but they do occur, generally in the most severe immediate cases. This conclusion, or impression, may be correct, based as it is on the antibody detection methodologies applied to date, but consistent failures to detect the antibodies may be due to the inadequacies or inappropriateness of those methodologies. Even with the small number of positive sera detected in some studies, reactions are often weak, quantitative inhibition results to demonstrate specificity are not shown, and details of the materials and methods used are not provided. The average association constant of IgE for contrast media was low in what appears to be the only study where the affinity of the reaction was looked at. The first more convincing demonstration of the detection of contrast media-reactive IgE antibodies involved activation of the hemisuccinate of ioxaglic acid to form the *N*-hydroxysuccinimide ester before linking to amino groups on human serum albumin as carrier protein (Fig. 10.2) and using the drug–carrier complex in immunoassays with patients' sera. Although binding uptakes were weak, specificity of the reaction with the contrast medium was demonstrated by dose-dependent inhibition in the range 25–80 % and IgE antibody was detected in 16 of 34 patients (47 %) with a history of adverse reactions to ioxaglate and in 14 of 68 patients whose sera were collected at the time of the adverse reaction to the contrast agent. The frequency of 47 % seems consistent with a previous claim of 42 % for the presence of IgE antibodies in patients with an adverse reaction to a contrast medium.

10.5.2.3 Basophil Activation Test

Unlike in vitro methodologies that detect binding of serum IgE antibodies, usually to an allergen in solid phase form, the basophil activation test (see Sect. 4.6) is an in vitro activation of a patient's basophils and as such the test mimics the interaction between the allergen and circulating basophils in the patient's body. The test is not, however, a primary diagnostic tool and is essentially complementary to skin tests and quantitation of allergen-reactive IgE antibodies. Despite references to its application to the investigation of contrast media adverse reactions, there currently appears to be very few published studies where it has been applied in this way. In a 2008 study the contrast media iomeprol and iopromide were diluted and used over a broad range up to 1 μg/ml and a minimum of 500 basophils per sample were activated (CD63+, IgE++) and assessed by flow cytometry. Because drugs give lower activation percentages than inhalant and venom allergens, activation was considered positive if data analysis showed more than 5 % activated basophils. In three patients the test revealed 15 % maximum activation of basophils at 1 μg/ml. Two patients showed positive results only with iomeprol while the third was positive to both contrast media. In the control samples, activation remained negative at all contrast media concentrations. A recent study in Thailand on 26 patients with diagnosed immediate reactions to contrast media and 43 healthy volunteers found significantly higher percentage activations at drug dilutions of 1:10 and 1:100 with the patients' than the volunteers' cells. Once again the conclusion was reached that the basophil activation test has potential as a diagnostic tool, especially as a confirmatory test.

10.5.3 Tests to Detect the Release of Mediators

From at least the early 1980s, studies relevant to the possibility of monitoring contrast media-induced histamine release either directly or indirectly via measurement of urinary methylhistamine have been pursued. Although concentrations of histamine and its metabolite have been shown to increase in some patients who had an adverse reaction to a contrast medium, diagnostic tests for these mediators have not often been used and it was not until an assay for tryptase (see Sect. 4.5.1) became widely available that routine measurement

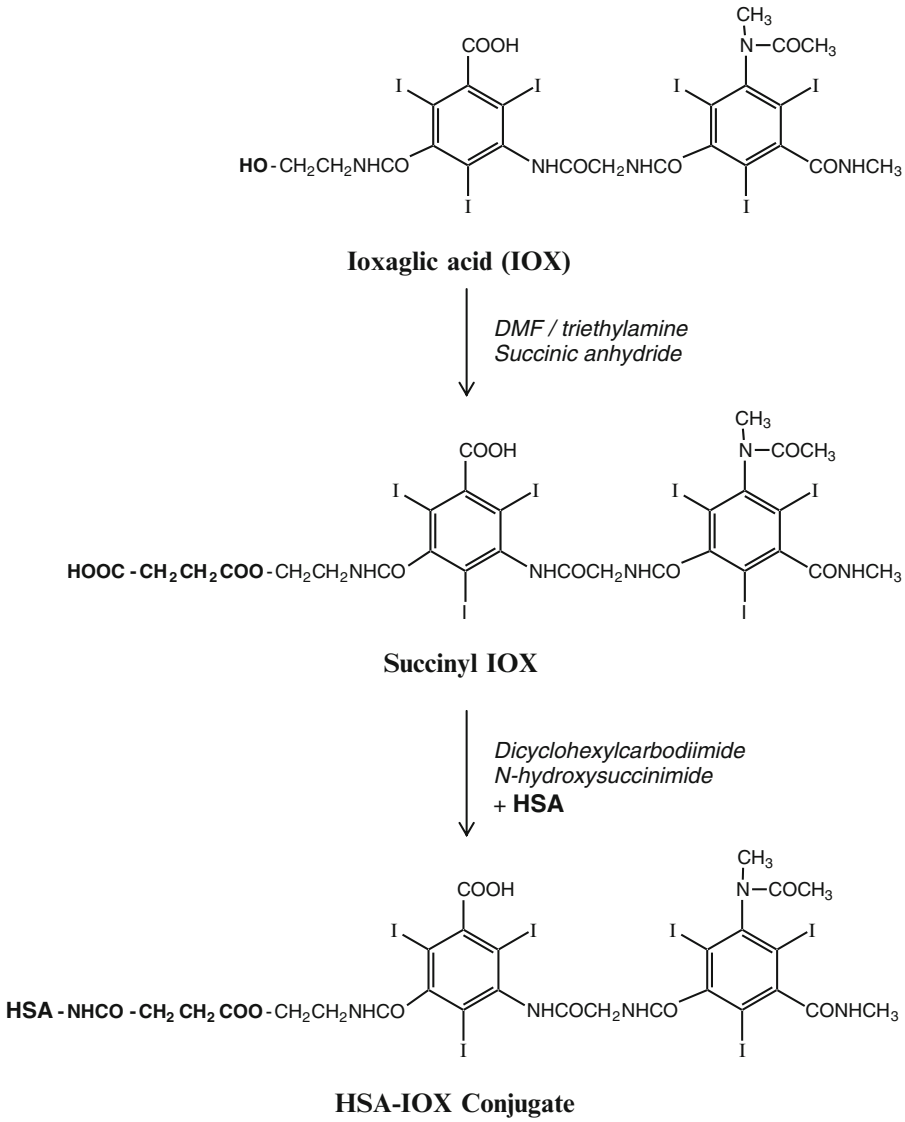


Fig. 10.2 Preparation of a drug–carrier complex of ioxaglic acid with human serum albumin for use in a solid phase assay for the detection of drug-reactive IgE antibodies. Structures in *bold* show reactive groups and point of attachment of albumin

of a mast cell mediator became almost standard practice. In one of the most informative studies, released histamine and tryptase levels correlated significantly with the severity of symptoms to contrast media and it was suggested that less clear results from many previous investigations may have been due to the recruitment of patients with minor or only moderate reactions. The relative half-lives of histamine (about 2 min) and tryptase (about 90 min) give another indication of why the latter mediator, the most abundant protein produced by the human mast cell, is preferred in diagnostic investigations of severe immediate but not delayed allergic reactions.

10.5.4 Challenge Tests

Although considered to be the "gold standard" in the diagnosis of drug hypersensitivity, challenge tests (Sect. 4.4) are time-consuming, are potentially dangerous, and tend to be reserved for use

Table 10.8 Challenge protocol for iodinated contrast media

Written informed consent obtained from each patient
Time interval should be at least 6 weeks since anaphylactic reaction
Patient observed during whole period of challenge
Emergency drugs and equipment available
Challenge doses increased stepwise at 30 min intervals up to a normal dose. Doses used: 0.05, 0.5, 1, 5, 7.5, 10, 25 ml. Total = 49.05 ml

From Trcka J et al. Am J Roentgenol. 2008;190:666

when the information gathered from other tests yields inconclusive results or contradictions. Challenge tests with contrast media have so far also been employed rarely but appear to be valuable to identify contrast media that are tolerated by challenging intravenously skin test-positive patients with skin test-negative agents. In addressing the paucity of data on the negative predictive value for skin tests with iodinated contrast media, 29 skin test-negative patients needing a new contrast medium were rechallenged without premedication. Mild reactions resulted in 1 of 24 patients with a history of an immediate reaction and one of four with a history of a non-immediate reaction, giving a negative predictive value of 96 %. Two patients with a positive skin test to an iodinated contrast medium tolerated an alternative drug without experiencing a reaction. A protocol for challenge with contrast media is set out in Table 10.8.

10.6 Premedication for the Prevention of Anaphylactoid/Anaphylactic Reactions to Iodinated Contrast Media

From the results of studies in the USA dating back to the mid-1970s, it was recommended that lower osmolality contrast media should be given to patients who had previously experienced what was called an "immediate generalized reaction." In addition, the prophylactic use of prednisone and diphenhydramine was recommended to reduce the chance of a reaction in high-risk patients. Such premedication is often given, but opinion of its effectiveness is divided and it could probably be said that the practice has not received wide support. There are many reports of breakthrough reactions—some detailing that hypersensitivity responses were not prevented in a number of patients; some reporting a significantly higher recurrence rate in those who had a previous mild reaction but prevention of a reaction in those who had a severe previous reaction; and a frequent finding that breakthrough reactions were of similar severity to the patients' initial reactions.

The theory behind the inclusion of histamine H_1 antagonists in the premedication is obvious but the mode of action of corticosteroids is not completely understood so some believe its inclusion cannot be explained and justified. Corticosteroids ultimately inhibit kallikrein, a peptide that lowers blood pressure and liberates bradykinin. Corticosteroids also act in the arachidonic acid cascade to inhibit the production of prostaglandins and leukotrienes, so there does seem to be some rationale for their use. There are some indications that premedication prevents the recurrence of many minor reactions. Some, or even many, of these reactions may not be immune mediated, proceeding instead via a nonspecific and low-level histamine release. In the case of severe immediate reactions, IgE antibody-mediated explosive histamine release from mast cells may overwhelm the potential effectiveness of premedication.

Despite calls to discontinue the prophylactic use of corticosteroids and antihistamines for contrast medium-induced anaphylactoid reactions in the USA, the recommendation for their use is still unaltered. This situation is in contrast to the attitude in some other countries, for example, France, but one wonders what the attitude of the critics of premedication would be if they faced injection of an iodinated contrast medium for angiography after experiencing a life-threatening reaction knowing they were allergic to both ionic and nonionic media of all osmolalities. Given our knowledge of the pharmacological effects of histamine H_1 antagonists and the steroids, could there be anything to lose in opting for premedication in such a situation? A suitable premedication regime is set out in the legend of Table 10.4.

Of course, for high-risk patients when administration of an iodinated contrast medium is regarded as essential, a nonionic agent would be

the first choice, but if that choice is also potentially dangerous and cannot be avoided, the patient should be informed of the risks, patient approval should be obtained, and resuscitation arrangements should be fully in place. Gadolinium-based contrast media may also be considered, but with these agents some extra factors need to be considered.

10.7 Gadolinium-Based Contrast Agents

Gadolinium, a rare earth metal, forms trivalent ions with paramagnetic properties that make solutions of chelated gadolinium complexes with large organic molecules useful as intravenously administered contrast agents detected by magnetic resonance imaging (MRI). Gadolinium-based contrast agents are approved by the FDA (and many other licensing authorities) for use with MRI as a contrast agent, but, although they can be used for magnetic resonance angiography (MRA), there is no approval for this use. The usual dose for many MRI applications is 0.1 mmol/kg up to a maximum approved dose of 0.3 mmol/kg for intravenous use. Above that figure the agents may induce potentially fatal nephrogenic systemic fibrosis (NSF), a scleroderma- and eosinophilic fasciitis-like disease of the joints, skin, eyes, and organs particularly in patients with kidney failure.

10.7.1 Molecular Structures of Gadolinium-Based Contrast Agents

These agents may be acyclic (linear) or macrocyclic and ionic or nonionic. Examples from each category are acyclic, ionic—gadopentetate (Gd-DTPA) dimeglumine; acyclic, nonionic—gadodiamide (Gd-DTPA-BMA); macrocyclic, ionic—gadoterate (Gd-DOTA) meglumine; and macrocyclic, nonionic—gadoteridol (Gd-HP-DO3A). The structures of the acyclic agent gadopentetic acid (gadopentetate dimeglumine is the salt) are shown in Fig. 10.3 and structures of three macrocyclics, gadoterate meglumine, gadoteridol, and the nonionic gadobutrol (Gd-BT-DO3A) are set out in Fig. 10.4. Stability of gadolinium chelates is a major concern and great emphasis has been placed on this because of the possibility of transmetallation, that is, exchange or release of free Gd^{3+}. With the Gd^{3+} caged within the chelate complex, the macrocyclic compounds are generally more stable than their acyclic counterparts.

10.7.2 Nephrogenic Systemic Fibrosis

Identified in 1997 and reported in 2000, nephrogenic systemic fibrosis (NSF) was first associated with gadolinium contrast agents in 2006. Chronic kidney disease, hepato-renal syndrome with renal insufficiency, and acute kidney injury were described as the clinical settings in early case reports and it soon became apparent that the disease affects multiple organs including the lungs, heart, liver, and muscles. In patients with reduced renal function, the prevalence of NSF after exposure to gadodiamide is reported to be 3–7 %. Of 589 patients who developed NSF associated with gadolinium contrast agents between 1997 and 2007, all were to linear chelates—68 % with gadodiamide, 26 % with gadopentetate, and 5 % with gadoversetamide. Subsequent to 2006, of 1,603 cases reported to the FDA, 93 % were from 60 hospitals in the USA and 4 % from 2 hospitals in Denmark. Gadodiamide was revealed to be a key factor in the relatively high incidence of NSF found in Denmark. The macrocyclic agents are regarded as low-risk compounds. By 2009, there had been no cases of NSF after exposure to a macrocyclic, but three cases were reported in Denmark in 2011.

10.7.3 Other Adverse Reactions

Risk factors for acute reactions to gadolinium-based contrast media and procedures and strategies to reduce those risks are summarized in Table 10.9. Note that the risk of an adverse reaction to a gadolinium-based contrast medium is eight times higher in patients who have experienced a previous reaction to a gadolinium agent. Gadolinium chelates have been used parenterally

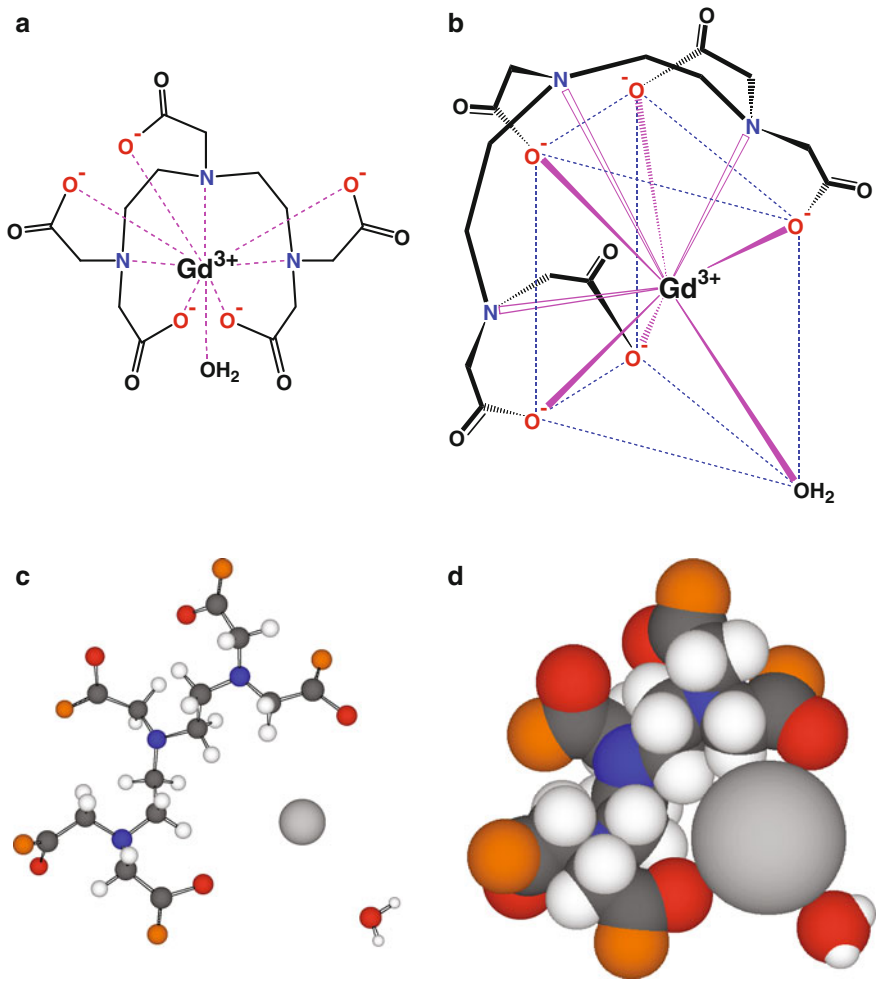
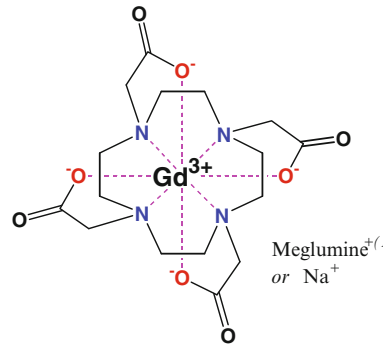
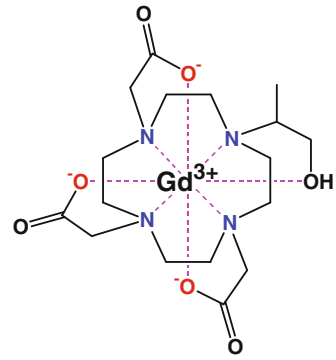
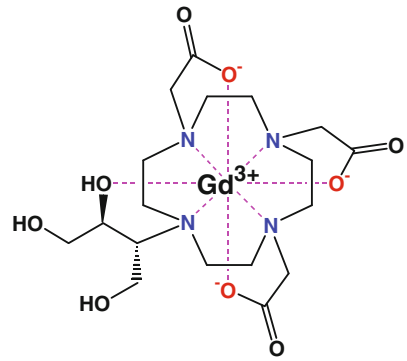


Fig. 10.3 2D structure (**a**, **b**), ball-and-stick (**c**), and CPK (**d**) models of gadopentetic acid, the first gadolinium-based magnetic resonance imaging contrast agent introduced in 1987. This linear, ionic agent is usually administered as the meglumine salt gadopentetate dimeglumine, a complex of Gd^{3+} with diethylenetriaminepentacetate ($DTPA^{5-}$) (Gd-DTPA) with the nine-coordinate Gd ion surrounded by three nitrogens and five oxygens and the ninth site occupied by a water molecule (**a**, **b**)

for over 30 years and are well tolerated in the vast majority of patients. Adverse reactions to gadolinium contrast media appear to be less frequent than reactions to the iodinated media. The American College of Radiology (ACR) Committee on Drugs and Contrast Media has reported that the frequency of all adverse events after injection of 0.1–0.2 mmol/kg of gadolinium chelate is in the range 0.07–2.4 % with the vast majority of these reactions being mild, for example, nausea, vomiting, headache, paresthesia, dizziness, itching, and coldness at the injection site. "Allergic-type" reactions have an even lower frequency of 0.004–0.7 % with symptoms of rash, urticaria, and rarely bronchospasm. Anaphylactic/anaphylactoid reactions are extremely rare (0.001–0.01 %). In one study, an adverse reaction rate of 0.48 % and an incidence of 0.01 % for severe anaphylactoid reactions were reported for gadolinium chelates. Of the 45 patients with 46 adverse reactions, 96 % were mild reactions, 2 % were moderate, and 2 % severe. Three (6.7 %) of the 45 patients had prior reactions to iodinated contrast media. In an

Macrocyclic-structure MRI contrast agent	*Structure*	*Trade name*
Gadoterate meglumine[(1)] *or* **sodium (Gd-DOTA)** *(ionic)*		Artirem, Dotarem
Gadoteridol (Gd-HP-DO3A) *(non-ionic)*		ProHance
Gadobutrol (Gd-BT-DO3A) *(non-ionic)*		Gadovist, Gadavist, Gadograft, Protovist

[(1)]Meglumine:

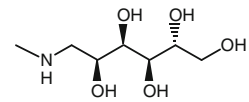


Fig. 10.4 Structures of three macrocyclic gadolinium-based magnetic resonance imaging contrast agents

Table 10.9 Risk factors for acute reactions to gadolinium-based contrast media[a] and procedures and strategies to reduce the risks[b,c]

Risk factors	
Patient related	Patient with history of: – Previous acute reaction to gadolinium contrast media – Asthma – Allergy requiring medical treatment
Contrast media related	Note: Risk not related to osmolality. Low dose makes osmolar load small
To reduce the risk	
For all patients	Keep patients in Radiology Dept. for 30 min after injection of contrast media Have drugs and equipment for resuscitation readily available[d]
For patients at increased risk of reaction	Consider alternative test not requiring a gadolinium agent Use a different gadolinium agent for previous reactors Consider premedication[e]

[a]Non organ specific
[b]Data adapted from ESUR Guidelines on Contrast Media. European Society of Urogenital Radiology, Version 7.0. View at http://www.esur.org/ESUR-Guidelines.6.0.html
[c]Risk of an acute reaction to gadolinium contrast media is significantly lower than the risk with an iodinated contrast media
[d]See Table 10.5
[e]See legend of Table 10.4 for a suitable premedication regime

assessment of the clinical safety and diagnostic value of the gadolinium chelate gadoterate meglumine in 24,308 patients injected with the agent, adverse reactions were seen in 0.4 % of the examinations and were mostly rated as minor, that is, feelings of warmth or taste alterations. Only one serious adverse reaction was seen. A review of 21,000 patients administered gadolinium contrast media in a Michigan hospital revealed 36 adverse reactions (0.17 %) classified into four groups: mild, nonallergic reactions (nausea, vomiting) 15 patients; mild reactions resembling allergy (hives, erythema, skin irritation) 12 patients; moderate reactions resembling allergy (respiratory symptoms) 7 patients; and life-threatening reactions resembling allergy (chest tightness, respiratory distress, periorbital edema) 2 patients (0.01 %). Reactions resembling allergy therefore occurred in 21 patients. Four of the patients had previous reactions to iodinated contrast media which is consistent with a previous conclusion that the risk of adverse reactions to gadopentetate is 3.7 times higher in patients with a history of reaction to iodinated contrast media. Gadopentetate dimeglumine was the agent most often implicated being the administered drug in 29 of the 36 patients (0.138 %) including both of the life-threatening reactions (0.01 %). These 2 % for gadopentetate differ significantly from an earlier investigation of the safety of the same agent where the overall incidence of adverse events was higher (1–2 %) while the incidence for anaphylactoid reactions was only 0.0003 %. For comparison, incidences of life-threatening reactions to iodinated contrast media are said to be 0.031 % for low-osmolarity agents and 0.157 % for high-osmolality agents.

A recent survey of the incidences of immediate reactions to gadolinium contrast media revealed rates of 0.2, 0.5, 1.2, and 3.3 per 1,000 injections of gadodiamide, gadopentetate dimeglumine, gadobenate dimeglumine, and gadoteridol, respectively. For the period 2004–2009, the FDA received reports of 40 NSF-unrelated deaths resulting from 51 million administrations of gadolinium contrast agents with incidences of 0.15, 0.19, 0.7, 0.97, and 2.7 per million for gadodiamide, gadoversetamide, gadoteridol, gadopentetate dimeglumine, and gadobenate dimeglumine, respectively. It has been pointed out that these figures represent a similar risk of death from traveling 86 miles by car! With gadoterate meglumine, positive skin tests, sometimes with a positive leukocyte histamine release test, indicated that the reactions were almost certainly IgE antibody mediated. Positive skin tests and a positive tryptase finding were also found in a case of anaphylaxis to gadobenate (Gd-BOPTA) dimeglumine. Skin testing with other gadolinium chelates generally revealed mono-sensitization with none of the other agents showing cross-reactions. In some of the investigations, skin test-negative findings with high concentrations of meglumine ruled it out as the provoking agent and direct and explosive mast cell degranulation by gadolinium chelates in the reported cases seems unlikely since in vitro experiments have shown that the concentrations needed for direct

histamine release are about 100–400 times the normal serum concentrations found in patients. The skin test findings so far with the gadolinium chelates suggest that this simple and easy-to-carry-out test might be useful for identifying alternative MRI contrast agents, but more extensive testing with many more patients is needed to validate the procedures before the predictive value of skin testing can be established. As with the iodinated contrast media, breakthrough reactions to gadolinium contrast agents, sometimes described as "allergic-like," have occurred after corticosteroid and antihistamine premedication.

In some cases where the history was reliable enough and where questioning about exposure to products containing gadolinium (for example, in CDs, electronic components, nuclear materials, alloys, phosphors, optical glass, ceramics, and manufacturing plants where the element is used) was undertaken, it became apparent that reactions to gadolinium chelates occurred on first exposure. As discussed before in this volume, this is not unusual in drug allergy and while a number of different speculative explanations have been offered for the mechanism of sensitization for some other drugs such as neuromuscular blockers, the advancement of any sort of plausible speculation accounting for allergic sensitization to complexes containing a rare earth metal seems even more difficult.

Summary

Iodinated Contrast Media

- Considering the very large number of administrations worldwide, iodinated contrast media are one of the safest of all drugs.
- Reactions to iodinated contrast media can be dose-dependent (toxic reaction) or unrelated to the dose (for example, an immunological reaction).
- Reactions range from a mild inconvenience such as heat sensation and nausea to a life-threatening emergency.
- In the great majority of cases reactions are mild and direct histamine release can account for the symptoms.
- Contrast media are better tolerated when the osmolalities of the injected media and body fluids are as close as possible. Nonionic media are better tolerated than the ionics.
- Adverse reactions are divided into acute or immediate and late or delayed. The former occur within an hour and the latter from about 1 h up to a week but usually 1–3 days.
- Acute reactions are conveniently divided into mild (with symptoms including nausea, vomiting, and headache), moderate (tachycardia/brachycardia, marked urticaria, severe vomiting), and severe (hypotensive shock, cardiac and respiratory arrest, laryngeal edema). Late reactions include nausea, vomiting, and especially (and usually self-limiting) maculopapular rash, exanthema, urticaria, and pruritus.
- Severe biphasic reactions to iodinated contrast media, that is, a life-threatening late reaction after an initial acute immediate reaction, are rare but can occur. After discharge following an acute immediate reaction to a contrast medium, patients should be made aware of the risk of a second-phase response.
- The incidence of acute (immediate) reactions to the ionic media is about 3–4 % with up to about 12 % reported. For the low-osmolar nonionic agents, the figure is 0.2–0.7 % (up to about 3 %). For severe immediate reactions (mainly anaphylactic), incidences are 0.1–0.4 % for ionic and 0.02–0.04 % for nonionic media. For very severe reactions the percentages drop to 0.04–0.004 %.
- Fatal reactions (1 in 100,000 to 1 in 170,000) are extremely rare and show no differences between low- and high-osmolar agents.
- Up to 80 % of reactions to an ionic agent can be avoided by substituting a nonionic medium.
- For delayed reactions, there is no difference between the incidences of reactions to ionic and nonionic media or between the different nonionic agents. Incidences of delayed reactions occurring in the first 24 h and over a 7-day period are approximately 4 % and 1–3 %, respectively.
- Risk factors: for immediate reactors—a previous immediate reaction to an iodinated contrast medium; bronchial asthma; use of

β-blockers; cardiac disease; highly allergic subject. For late reactors–a previous reaction; treatment with IL-2; a history of drug allergy; contact allergy.

- Evidence that immediate reactions to a contrast medium is IgE antibody mediated is based largely on skin test results with or without tryptase measurements and very occasionally serum IgE and basophil activation tests.
- Delayed reactions mainly manifest as exanthematous skin eruptions. They are mediated by antigen-specific effector T cells with as yet poorly defined cytokine involvement.
- Contrast media-induced hypersensitivity has traditionally been regarded as nonallergic in nature with skin testing not relevant. Skin tests with iodinated contrast media are, however, positive in a subgroup of reactors.
- In a large multicenter study, 50 % of immediate reactors tested within 2–6 months of the reaction showed a positive skin test. The prick test was only rarely positive. The intradermal test was clearly more informative.
- For delayed reactors, a combination of intradermal and patch testing identified the maximum number of positive reactions (47 %). The highest number of positives was detected when tests were performed within 6 months of the reaction.
- Cross-reactions between iodinated contrast media were detected in skin tests on delayed reactors making intradermal and patch tests useful tools for selecting a safe contrast medium.
- IgE antibodies to iodinated contrast media have not been consistently and convincingly demonstrated. This raises doubts about patient selection (that is, the degree of severity of patient reactions) and the adequacy and appropriateness of the present IgE test methodologies.
- Challenge tests with contrast media, rarely employed and refused by most patients, are valuable for the identification of tolerated skin test-negative agents.
- Opinion on the effectiveness of premedication with corticosteroids and a histamine H_1-antagonist is divided since many breakthrough reactions have been reported.
- A significant number of immediate and delayed reactors with positive skin tests to contrast media reacted on first exposure to the agents.

Gadolinium-Based Contrast Agents

- Gadolinium, a rare earth metal, forms ions with paramagnetic properties making the toxic metal in chelate form a useful contrast agent that can be detected by magnetic resonance imaging.
- Gadolinium-based contrast agents were associated with nephrogenic systemic fibrosis in 2006. The linear chelates, and especially gadodiamide, are most commonly implicated. The macrocyclic agents are regarded as relatively safe.
- Gadolinium chelates show an adverse reaction incidence of about 0.48 % and an incidence of about 0.01 % for anaphylactoid reactions. This is lower than the corresponding figures for iodinated contrast media.
- There are a number of reports of anaphylaxis to gadolinium-based contrast media with the diagnosis supported in some cases by positive skin tests to the agents.
- Some adverse reactions occurred on first exposure to the contrast agents.
- Gadolinium contrast media are well tolerated by the vast majority of patients and are regarded as remarkably safe drugs.

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Biologics

11

Abstract

Currently, ~28–30 mAbs are approved or under consideration for approval as specific therapies in the USA or European Union, although about 350 new mAbs for therapeutic application in humans are in the commercial pipeline. So far, the number of target antigens for the mAbs is surprisingly small with more than one of the approved antibodies specific for TNF, HER2, CD20, EGFR, or VEGF. Other specificities include EpCAM, glycoprotein IIb/IIIa, CD30, CD52, C5, α-4 integrin, IgE, IL-6R, BLys, IL-1β, and RANK-L. Initial infusion reactions to some mAbs may provoke tumor lysis syndrome, cytokine release syndrome, and systemic inflammatory response syndrome. Systemic and cutaneous reactions also occur to mAbs. Rituximab, for example, may cause serum sickness, vasculitis, cutaneous reactions, interstitial pneumonitis, and ARDS as well as post-infusion reactions. Some patients receiving cetuximab experienced severe immediate hypersensitivity reactions. The antibodies involved are IgE specific for α-D-galactose-(1–3)-β-D-galactose and reactive with this disaccharide present on the Fab portion of the chimeric antibody. The nature of, and main adverse reactions to, etanercept, the synthetic IFNs pegylated IFNα-2a and pegylated IFNα-2b, IL-2, denileukin diftitox, anakinra, aflibercept, anti-thymocyte globulin, epoetins, and recombinant human insulin are discussed.

Until the late 1990s, the backbone of treatments for malignancies was the administration of cytotoxic chemotherapeutic agents. However, from about 1998 and with ever expanding usage over the last 14 years, passive immunotherapy with targeted agents, generally monoclonal antibodies, is being used to manage various human cancers, some autoimmune diseases such as rheumatoid arthritis and Crohn's disease, some cardiovascular diseases, systemic lupus erythematosus, asthma, and for the prevention of organ transplant rejection. This new class of therapeutic drug, often referred to simply as "biologics," encompasses an expanding diversity of agents, many of which are genetically engineered copies or modifications of natural products of the body's immune system. Biologics may be composed of proteins, nucleic acids, sugars, or combinations of these; they may

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_11, © Springer Science+Business Media, LLC 2013

be of human, animal, or microbial origin; and, in the vast majority of cases, they are created by biological processes, or biotechnology, rather than chemical synthesis. Besides recombinant therapeutic proteins such as some antibodies, cytokines, and receptors, included in any list of biologics would be gene therapies, somatic cells, adult and embryonic stem cells, vaccines, tissues, and blood products and components.

11.1 Monoclonal Antibodies for Therapy

Monoclonal antibodies (mAbs) for the treatment of diseases belong to a new class of therapeutic agents called biologics. From their announcement in the scientific literature in 1975 and for many years thereafter until more recent times, these antibodies have been produced using mouse hybridoma cells prepared by fusing spleen cells from an immunized mouse with mouse myeloma cells. The hybridoma cells so produced retain the capacity to make specific antibody while the myeloma cells impart the capacity of the cells to grow indefinitely in culture, continuously secreting antibody. For therapeutic use in humans, the mouse antigens rapidly induce an immune response which not only inhibits the mouse antibody's action but can also provoke allergic reactions including anaphylaxis. This has led increasingly to the development of methods to humanize mAbs. One approach involves the production of chimeric antibodies by splicing the variable regions encoding the antigen-recognition determinants from a mouse antibody into the constant and Fc regions of human IgG. While this is one step forward in attempts to eliminate or attenuate an immune reaction, mouse antigens in the variable regions can sometimes still stimulate an immune response. Another approach involves the creation of a phage display library by the fusion of gene segments encoding human antigen-binding variable regions to genes encoding bacteriophage protein coat. Newer methods for the production of human mAbs utilize transformed human B cells, plasmablasts secreting antibody, or by generating human B cell hybridomas.

11.1.1 Nomenclature

The nomenclature of monoclonal antibodies is the one used by both the U.S. Adopted Names (USAN) and World Health Organization's International Proprietary Names (INN) for pharmaceuticals. All of the monoclonal antibody names end with the stem *-mab* and use preceding substems depending on the structure and function of the antibodies. Different modified suffixes or stems are added to distinguish the origins of mabs. Those of murine origin are designated by the stem *-omab*; chimeric antibodies in which the variable region is spliced into a human constant region are given the *-ximab* stem; humanized antibodies with the murine hypervariable regions spliced into a human antibody have the *-zumab* stem and antibodies with a complete human sequence are given the *-mumab* or *-umab* suffix.

11.1.2 Monoclonal Antibodies Approved for Therapy

Only 11 years after the publication of Köhler and Milstein's paper on the development of hybridoma technology, the first therapeutic monoclonal antibody OKT3 (muromonab, Orthoclone) received regulatory approval. Over the last 15 years, specially designed and produced mAbs have become one of the most important and successful therapies for patients with hematological malignancies and solid tumors. These targeted agents are also finding increasing application for the treatment of other diseases such as chronic asthma, psoriasis, systemic lupus erythematosus, and macular degeneration; the prevention of the rejection of transplanted organs and graft-versus-host reactions; inhibition of platelet aggregation in some cardiovascular diseases; treatment of autoimmune diseases such as Crohn's disease and rheumatoid arthritis; for paroxymal nocturnal hemoglobinuria and cryopin-associated periodic syndrome; and for inhibiting respiratory syncytial virus. Most are produced in Chinese hamster ovary cells, Sp2/0 cells, or NSO cells. The subclass of immunoglobulin used in therapeutic mAbs is an important consideration, especially in

treating tumors. A glance at a list of mAbs shows that IgG1 is frequently selected, IgG2 and IgG4 are occasionally employed, and IgG3 is rarely if ever used. This relates to the different biological properties of the antibody subclasses—IgG1 is the subclass of choice for antibody-dependent cell-mediated cytotoxicity which makes it eminently suitable for treating cancer cells while IgG4, which does not aid cytotoxicity, is the choice when cell killing is not wanted. IgG3 is seldom used since it has a significantly decreased half-life.

At the time of writing, the ~28–30 mAbs approved or under consideration for approval as specific therapies in the USA or European Union are listed in Table 11.1. The great majority of these mAbs are for cancer immunotherapy. Humanized and human monoclonal antibodies each comprise about one-third of the approved total. Two of the antibodies are administered with radiolabels, ibrutumomab tiuxetan with yttrium-90 or indium-111 and tositumomab with ^{131}I; catamaxomab has dual specificity, for EpCAM (epithelial cell adhesion molecule) and CD3; and only one, brentuximab vedotin, is an antibody–drug conjugate. However, it is true to say that the number of target antigens is surprisingly small with more than one of the approved antibodies specific for TNF, HER2, CD20, EGFR, or VEGF. It is said that about 350 new mAbs for therapeutic application in humans are in the commercial pipeline and it is likely that many of these will be antibody–drug conjugates, bispecific, and/or specifically engineered fragments or domains. There may be at least some truth in those who predict that the future of therapy belongs to the emerging biologics.

11.1.3 Immune Reactions to Monoclonal Antibodies

Reactions, both immune and innate and to human as well as foreign proteins, may occur to mAbs. Acute reactions caused by a number of different mechanisms have been reported. These reactions include true, type I anaphylaxis, delayed reactions, anaphylactoid responses, serum-sickness-like reactions, cytokine release syndrome, and tumor lysis syndrome (refer Sect. 13.5). The range of clinical manifestations include those seen in local skin reactions at the injection site through to cutaneous and systemic hypersensitivities and sometimes pyrexia, an influenza-like syndrome, and the potentially fatal systemic inflammatory response syndrome. Acute anaphylaxis and anaphylactoid reactions are well known to occur with mAbs. Anaphylaxis to some mAbs such as cetuximab and omalizumab has been frequently described and is of special interest (see below), but there are reports of reactions to others including muromonab, basiliximab and OKT3. Incidences of immediate hypersensitivity have been reported for infliximab (2–3 %), rituximab (5–10 %), trastuzumab (0.6–5 %), omalizumab (0.1–0.2 %), and from <1 to 3 % for cetuximab with much higher rates reported in some regions of the USA (see Sect. 11.1.3.2). Serum sickness has been described for a number of mAbs including the chimeric antibodies infliximab and rituximab and the humanized mAbs alemtuzumab and natalizumab. For both the initial reactions and overall, cutaneous reactions are the most frequently seen adverse responses to mAbs.

Reactions following initial infusions of antibody are common, but these can usually be handled by a cautious rate of infusion, appropriate hydration and diuresis, and, if necessary, premedication. Twenty six percent of initial reactions are reported to be mild, 48 % moderate, and 26 % severe. The initial infusion reaction to some mAbs, for example, rituximab (see below), may provoke tumor lysis syndrome, cytokine release syndrome, and systemic inflammatory response syndrome. Tumor lysis syndrome, noted particularly with rituximab, can occur following cancer treatment and sometimes without treatment. It is believed to be the result of breakdown products of cancer cells leading to increased levels of some metabolites and reflected in conditions such as hypercalcemia, hyperkalemia, hyperphosphatemia, acute uric acid nephropathy, and acute renal failure. The syndrome can occur in the early stages of mAb therapy and is potentially life-threatening. Cytokine release syndrome, also called cytokine storm, is commonly seen after

Table 11.1 Therapeutic monoclonal antibodies (mAbs) marketed or under review in the USA or European Union (As at June 2012)

Generic name	Type of mAb	Cell line	Target[a]	Mechanism of action	Approved indication	Trade name
-omabs						
Catumaxomab	Rat IgG2b/Mouse IgG2a bispecfic	Hybrid hybridoma	EpCAM/CD3	Binds both EpCAM and CD3	Malignant ascites	Removab®
Ibritumomab tiuxetan	Murine IgG1κ	CHO	CD20	Binds malignant B cells[b]/ADCC[c] and CDC[c]	Non-Hodgkin lymphoma	Zevalin®
Tositumomab-^{131}I	Murine IgG2aλ	Hybridoma	CD20	Kills B cells with ^{131}I	Non-Hodgkin lymphoma	Bexxar®
-ximabs						
Abciximab	Chimeric IgG1κ Fab	Sp2/0	Glycoprotein IIb/IIIa	Platelet aggregation inhibitor	Cardiovascular disease	Reopro®
Basiliximab	Chimeric IgG1κ	Sp2/0	α chain IL-2 receptor (CD25)	Reduces T cell activation	Prevent organ transplant rejection	Simulect®
Brentuximab vedotin[d]	Chimeric IgG1κ[d]	CHO	CD30	Antimitotic MMAE[d]	ALCL and Hodgkin lymphoma	Adcetris®
Cetuximab	Chimeric IgG1κ	Sp2/0	EGFR[e]	Turns off cell division	Colorectal and head and neck cancers	Erbitux®
Infliximab	Chimeric IgG1κ	Sp2/0	TNF	Inhibits TNF-α	Autoimmune disease (RA, Crohn's)	Remicade®
Rituximab	Chimeric IgG1κ	CHO	CD20	B cell killing	Non-Hodgkin lymphoma	MabThera® Rituxan
-zumabs						
Alemtuzumab	Humanized IgG1κ	CHO	CD52	Eliminates lymphocytes	Chronic lymphocytic leukemia	Campath-1H®
Bevacizumab	Humanized IgG1κ	CHO	VEGF[f]	Angiogenesis inhibitor	Colorectal cancer	Avastin®
Certolizumab pegol[g]	Humanized IgG1κ Fab, pegylated[g]	*E. coli*	TNF	Inhibits TNF-α	Crohn's and RA	Cimzia®
Eculizumab	Humanized IgG2/4κ	NSO	C5[h]	Inhibits cleavage of C5	Paroxymal nocturnal hemoglobinuria	Soliris®
Natalizumab	Humanized IgG4κ	NSO	α-4 integrin[i]	Blocks lymphocyte trafficking	MS and Crohn's	Tysabri®
Omalizumab	Humanized IgG1κ	CHO	IgE	Removes IgE	Chronic asthma	Xolair®
Palivizumab	Humanized IgG1κ	NSO	RSVF[j]	Inhibits virus entering cell	RSV	Synagis®
Ranibizumab	Humanized IgG1κ Fab	*E. coli*	VEGF[f]-A	Angiogenesis inhibitor	Macular degeneration	Lucentis®
Tocilizumab	Humanized IgG1κ	CHO	IL-6R	Blocks IL-6-induced inflammation	RA	Actemra®
Trastuzumab	Humanized IgG1κ	CHO	HER2[k]	Prevents over expression of HER2	Breast cancer	Herceptin®
-(m)umabs						
Adalimumab	Human IgG1κ	CHO	TNF	Inhibits TNF-α	Autoimmune disease (RA, Crohn's)	Humira®
Belimumab	Human IgG1λ	NSO	BLys[l]	Inhibits immuno-stimulant BLys	Systemic lupus erythematosus	Benlysta®

Canakinumab	Human IgG1κ	Sp2/0	IL-1β	Blocks inflammation by IL-1β	CAPS[m]	Ilaris®
Denosumab	Human IgG2κ	CHO	RANK-L	Inhibits activation of osteoclasts by RANK-L	Bone loss	Prolia® Xgeva®
Golimumab	Human IgG1κ	Sp2/0	TNF	Inhibits TNF-α	Autoimmune disease	Simponi®
Ipilimumab	Human IgG1κ	CHO	CTLA-4[n]	Blocks interaction of CTLA-4 with its ligands[o]	Metastatic melanoma	Yervoy®
Ofatumumab	Human IgG1κ	NSO	CD20	B cell killing	Chronic lymphocytic leukemia	Arzerra®
Panitumumab	Human IgG2κ	CHO	EGFR[e]	Turns off cell division	Colorectal cancer	Vectibix®
Ustekinumab	Human IgG1κ	Sp2/0	IL-12, IL-23	Inhibits inflammation by IL-12, IL-23	Psoriasis	Stelara®
Pending						
Pertuzumab	Humanized IgG1κ	CHO	HER2[k]	Inhibits dimerization of HER2 with other HER receptors[p]		
Raxibacumab	Human IgG1κ	NSO	*B. anthracis* protective Ag	Antitoxin activity. In review		

RA rheumatoid arthritis. *CHO* Chinese hamster ovary cells; Sp2/0, BALB/c mouse spleen cells fused with P3 myeloma. Cells do not secrete Ig, are resistant to 8-azaguanine, and are HAT sensitive. NSO, non-Ig secreting, non-L-chain synthesizing. 8-Azaguanine-resistant and HAT-sensitive mouse myeloma cell line

[a]Specificity of mAb

[b]With Yttrium-90 or Indium-111

[c]*ADCC* antibody-dependent cell-mediated cytotoxicity, *CDC* complement-dependent cytotoxicity

[d]Conjugated to the cytotoxic agent monomethyl auristatin E (MMAE)

[e]*EGFR* epidermal growth factor receptor

[f]*VEGF* vascular endothelial growth factor (a subfamily of growth factors; includes VEGF-A)

[g]Attached to PEG (polyethylene glycol)

[h]Complement component 5

[i]α-4 integrin (CD49d)

[j]*RSV* Human respiratory syncytial virus; F (viral protein coat antigen)

[k]*HER2* human epidermal growth factor receptor. Also known as Neu, ErB-2, CD340, or p185

[l]*BLys* B lymphocyte stimulator; B-cell activating factor, BAFF

[m]*CAPS* cryopyrin-associated periodic syndrome; Muckle–Wells syndrome

[n]*CTLA-4* cytotoxic T-lymphocyte antigen 4; CD152

[o]Ligands for CTLA-4—CD80/CD86

[p]Approved by FDA June 2012 for metastatic breast cancer. Trade name, Perjeta®

infusions of anti-immune cell mAbs (again, such as rituximab). It is thought to be a consequence of antibody binding to, and activation of, the cells producing a systemic inflammatory response together with high fever. The reaction, which is similar in some respects to infection, can induce life-threatening pulmonary edema and possibly death. Systemic inflammatory response syndrome affects the whole body and resembles the response seen to sepsis. It may lead to respiratory distress syndrome, renal failure, gastrointestinal bleeding, and dysfunction of the central nervous system.

Although there are many reports of adverse reactions, especially infusion reactions, to many of the mAbs in current use, most information is available on five widely and frequently used antibodies and they will therefore be considered in some detail. Reactions seen to these four mAbs, ranging from mild skin rashes to full-blown anaphylaxis and including infusion reactions, are similar to those seen with the other antibodies.

11.1.3.1 Omalizumab

Omalizumab, a humanized IgG1κ mAb with specificity for human IgE antibodies (Table 11.1), is approved for the treatment of severe allergic asthma in patients 12 years or older. It binds to free, circulating IgE antibodies and membrane-bound IgE molecules on some cells such as B lymphocytes expressing the antibody, but it does not bind to IgE already bound to mast cells, basophils, and dendritic cells. This selectivity of binding results from omalizumab binding to a determinant in the Cε3 region of the free antibody, the same region involved in binding to the FcεRI receptor on the mast cell. Interference with binding due to steric hindrance also occurs when IgE is bound to the receptor, in this case preventing the binding of the mAb to the patient's IgE molecules. Omalizumab has proven extremely efficient in depleting free circulating IgE to almost negligible levels with two interesting consequences. As IgE levels are reduced, the complementary receptors on mast cells, basophils, and dendritic cells fall correspondingly and this has the consequence of rendering the cells less sensitive to allergen stimulation. Secondly, antigen trapping by IgE and subsequent presentation by dendritic cells are markedly reduced or prevented resulting in no further activation of allergen-specific Th2 cells.

Despite the clear efficiency of omalizumab in reducing levels of IgE antibodies, the question of its safety remains paramount. In one assessment of the mAb's safety and tolerability, data from completed clinical studies involving more than 7,500 patients with asthma, rhinitis, and other conditions were reviewed with a focus on hypersensitivity reactions, other immune effects, thrombocytopenia, malignant neoplasia, and parasitic infections. Findings revealed that omalizumab had good safety and tolerability records that were maintained for up to 4 years in one study. The incidence of anaphylaxis to the agent was 0.14%, twice the figure seen in control patients, but based on an estimated exposure of 57,300 patients, the frequency of anaphylaxis was estimated to be at least 0.2 %. Increased risks of malignant neoplasia and thrombocytopenia were not detected. A review undertaken by the U.S. Food and Drug Administration Adverse Event Reporting System of cases of anaphylaxis to omalizumab for the period June 2003 and December 2006 revealed 124 cases, many of whom experienced delayed (>2 h) onset of the reaction or protracted progression of the symptoms. A similar review for the period June 2003 to December 2005 carried out by a joint task force of the major U.S. allergy societies found 41 cases of anaphylaxis in 35 of 39,510 patients given omalizumab, a rate of 0.104 %. These figures show that in 2006, another 83 episodes of anaphylaxis were recorded and this finding, together with the higher incidence of anaphylaxis in the post-marketing period than in the pre-marketing clinical trials, led the Omalizumab Joint Task Force to issue guidelines for the administration of the agent. The Task Force recommended: (1) Prior to administering omalizumab, patients should be assessed for vital signs, asthma control, and lung function; (2) Informed consent should be obtained; (3) Patients should be advised how to recognize anaphylaxis, how to use an epinephrine auto-injector and to

ensure that the injector is always available during the administration of omalizumab; (4) Omalizumab should only be administered in a facility that has both the staff and equipment to treat anaphylaxis; and (5) Patients should be observed for 2 h after each of the first three injections and for 30 min after subsequent injections of the mAb.

11.1.3.2 Cetuximab

Cetuximab, a chimeric mouse-human IgG1κ mAb to the epidermal growth factor receptor (EGFR) used to treat colorectal cancer and squamous cell cancer of the head and neck, has been shown to be associated with anaphylaxis in a most curious way. Natural antibodies, mostly of the IgG class, specific for an α-1,3-linked D-galactose disaccharide, a structure found in many animals but not humans, are found in all individuals as a result, it is thought, of inactivation of the gene for the enzyme α-1,3-galactosyltransferase. Presumably, this resulted in the loss of immune tolerance to the α-D-galactose determinant and the production of antibodies to it. Although the biological role of this antibody remains unclear, it may provide some protection against gastrointestinal bacteria and contribute to the removal of senescent red cells via recognition of cryptic α-D-galactosyl residues exposed in the course of cell aging. Humans of blood group B have a terminal α-1,3-linked-d-galactose on their blood group substances in secretions and on red cells, but the presence of a penultimate α-1,2-linked L-fucose prevents binding to the antibody. By the early 1990s it was known that the anti-D-galactosyl antibodies in humans were potentially capable of interacting with therapeutic recombinant proteins expressing the complementary α-linked determinant and this became a reality just a few years ago when patients receiving cetuximab experienced severe immediate hypersensitivity reactions. The antibodies involved were found to be IgE, specific for α-D-galactose-(1–3)-β-d-galactose and reactive with this disaccharide present on the Fab portion of the chimeric antibody at asparagine 88 of the heavy chains. Most of the patients who reacted already had the IgE antibodies in their serum before administration of cetuximab, but how they became sensitized to the disaccharide in the first place remains uncertain. A possible explanation was suggested by the occurrence in patients given cetuximab of delayed-onset anaphylaxis, angioedema, and/or urticaria 3–6 h after consuming red meat. Following some investigations and questioning of patients in a large area of the U.S. South East, it has been speculated that IgE antibodies to the α-linked D-galactose disaccharide present in the meat may be linked to prior tick bites. Previous results by Van Nunen and colleagues in Sydney who reported an association between reactions to tick bites and allergy to red meats appear to support this suggestion. The mechanism of this association is, as yet, unknown and is itself subject to speculation. An anti-cetuximab IgE ELISA was recently reported with the claim that it could be a valuable test to identify potential cases of anaphylaxis following cetuximab infusion. Cetuximab-reactive IgE antibodies were detected in 24 of 92 (26.1 %) of pretreatment patients and 33 of 117 (28.2 %) healthy blood donors. Hypersensitivity reactions occurred in 14 of the 92 patients (15.2 %) and 8 of these were grade 3–4 reactions. Seven of the eight patients (87.5 %) with severe hypersensitivity reactions had anti-cetuximab IgE antibodies while 14 of 78 (17.9 %) with no signs of hypersensitivity showed the presence of antibodies.

With the greatly expanding administration of biologic agents, it is already clear that infusion reactions occur frequently, and because many patients being treated with mAbs often have few or even no other therapeutic alternatives, successful desensitization to mAbs is likely to be increasingly sought. Claim of a successful desensitization protocol to cetuximab has been published. The patient was premedicated with prednisolone 12 h and 1 h before desensitization and with diphenhydramine 30 min before desensitization. Beginning with an infusion dose of 0.001 mg of cetuximab, doses were doubled every 15 min with each dose tolerated until a total of 64 mg of cetuximab was reached. The appearance of a pruritic cutaneous reaction was managed with diphenhydramine, a 30 min waiting period, and dose and infusion rate reductions. The final dose

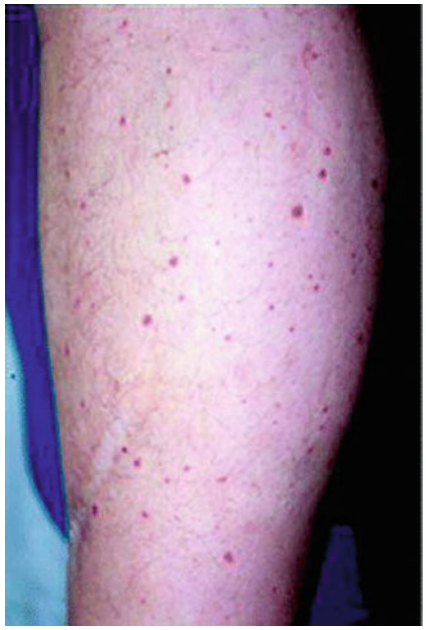

Fig. 11.1 Palpable purpura in a patient with hypersensitivity vasculitis (leukocytoclastic vasculitis), a small vessel vasculitis usually involving post-capillary venules in the dermis. This cutaneous manifestation of vasculitis occurs occasionally following treatment with mAbs and some other biologic agents (Photograph of Dr. John Stone) (Reproduced with permission from Weyand CM, Goronzy J, in Klippel JH, Stone JH, Crofford LeJ, White PH, editors. Primer on the Rheumatic Diseases, 13th ed. New York: Springer, 2008)

of cetuximab (325 mg) was tolerated giving a cumulative dose of 844 mg. When challenged with cetuximab 1 week later, the patient tolerated the dose without difficulty. This was expected since the half-life of cetuximab is approximately 4 days. It was predicted that the protocol might be useful to many patients.

11.1.3.3 Infliximab

Like cetuximab, infliximab is a mouse-human chimeric IgG1κ mAb. With specificity for TNF, the antibody is used to treat autoimmune diseases such as Crohn's disease and rheumatoid arthritis (Table 11.1) and there are a few reports of it inducing rapid recovery of lesions in several cases of toxic epidermal necrolysis (Sect. 3.6.3.7). A variety of reactions, both systemic and cutaneous, have been reported following administration of infliximab. These include maculopapular rashes, urticaria, psoriasis, flare-up of atopic dermatitis, leukocytoclastic vasculitis (Fig. 11.1), serum sickness, and anaphylaxis. In a large center study of 165 consecutive patients who received 479 infliximab infusions, the overall incidence of infusion reactions to the mAb was 6.1 % with 9.7 % of the patients affected. Mild, moderate, and severe reactions occurred in 3.1, 1.2, and 1 % of infliximab infusions, respectively. Serum tryptase levels suggested that acute reactions in 11 of 14 patients were not type I hypersensitivity reactions. Delayed reactions were rare, resulting from only 0.6 % of infusions. An examination of incidences of systemic and delayed reactions to infliximab in children as well as adults with Crohn's disease revealed that 14 % of 86 patients experienced severe systemic reactions from a total of 304 infusions. A significant difference between the results for adults and children was noted—severe systemic reactions were seen in 11 of 52 adults (21.2 %) and in only one of 34 (2.9 %) children. These reactions were characterized by hypotension, mucosal irritability, and laryngospasm requiring epinephrine, antihistamines, and/or corticosteroids. Delayed reactions, which manifested as arthralgia, fever, and myalgia requiring corticosteroids were seen in eight adults (9.3 %); no delayed reactions occurred in children. An examination of the extent of, and reasons for, discontinuation of infliximab treatment in 84 patients with established rheumatoid arthritis revealed that 28 (33 %) discontinued the therapy. The main reason for discontinuation was an adverse reaction in 16 of the 84 patients (19 %). In this group, 9 of the 16 patients (10.7 %) experienced an immediate hypersensitivity reaction. There are many reports of immediate reactions, in particular anaphylaxis, to infliximab. From FDA and other sources, over 650 anaphylactic reactions to the mAb at an incidence of just under 0.9 % occurred in the period 1999–2012. Most reactions (nearly 70 %) occur during the first month of therapy; females account for approximately 60 % of reactors and children under 10 years of age for 7.5 % of reactors.

Cutaneous reactions may also be provoked by infliximab. Three patients with rheumatoid arthritis, none of whom had a personal or family history of psoriasis, developed what was described as psoriasiform skin lesions 6–9 months after the initiation of infliximab therapy. Two of the

patients developed palmoplantar pustular lesions and scaly plaques on the extremities, while the third patient had erythematous plaques with silvery white scales on the scalp. Other rare adverse reactions to infliximab that may have an immunological basis include demyelinating polyneuropathies, peripheral neuropathy, drug-induced lupus, and hepatitis. A combination of the latter two conditions has been reported with the use of infliximab for psoriasis.

Successful desensitizations have been reported in adult and child patients who experienced an anaphylactic/anaphylactoid reaction to infliximab. Based on the adult patient's weight of 70 kg and the standard dose of 5 mg/kg, a total infliximab infusion of 353 mg divided into 11 increments was given IV every 15 min over a 4 h period with a starting dose of 3 μg and a final dose of 160 mg. For a 10-year-old male child, the schedule was the same except for the lower dosages (total of 208 mg), increments ranging from 2 μg to 80 mg and a final dose of 80 mg. For both patients, IV infliximab was tolerated without incident and each experienced clinical improvement over the subsequent 2 months of treatment.

11.1.3.4 Adalimumab

Like infliximab, adalimumab is a mAb targeted at TNF and used for Crohn's disease and rheumatoid arthritis, but, unlike infliximab, it is a fully human mAb of the IgG1κ class and can be administered subcutaneously. Being a possible substitute for infliximab in patients intolerant to that mAb, in 2004 adalimumab was examined for safety and efficacy in seven patients who had experienced immediate or delayed hypersensitivity reactions to infliximab and one patient with infliximab-induced lupus. Adalimumab proved to be well tolerated leading to the conclusion that it might prove to be a safe and effective substitute for patients allergic or otherwise intolerant to infliximab, In subsequent years, a range of different apparent hypersensitivity reactions induced by adalimumab have become apparent. These include psoriasis, exacerbation of palmoplantaris pustulosa psoriasis, asthma, bronchospasm, autoimmune hepatitis, and a number of different skin reactions, some severe. Like infliximab, adalimumab has been reported to elicit psoriasiform plaques on elbows, arms, and thighs together with palmoplantar pustular lesions. More severe cutaneous reactions to the mAb also occur such as erythema multiforme-like skin reaction with papulopustular exanthema at the injection site and on the palms and soles followed by skin desquamation and at least two cases of Stevens–Johnson syndrome. Systemic reactions to adalimumab have been reported. In one case, a patient with spondylarthritis treated with the mAb experienced two reactions consisting of generalized itching, angioedema of the lips, dizziness, and visual disturbances. Skin prick and intradermal tests with adalimumab produced strong immediate positive reactions, but serum IgE antibodies were not detected using a specially prepared adalimumab Phadia solid phase.

11.1.3.5 Rituximab

Rituximab, like cetuximab and infliximab, is a *-ximab* chimeric IgG1κ mAb (Table 11.1). It contains murine light and heavy chain variable region sequences and human constant region sequences. Administered for non-Hodgkin lymphoma, rituximab is targeted to CD20 (human B lymphocyte-restricted differentiation antigen Bp35), a B lymphocyte antigen involved in the development and differentiation of B cells into plasma cells. CD 20 is found on B cell lymphomas, B cell chronic lymphocytic leukemia, hairy cell leukemia, and melanoma cancer stem cells and is expressed on more than 90 % of B cell non-Hodgkin lymphomas but not on normal plasma cells, hematopoietic stem cells, or other normal tissues. In 1997 rituximab was the first mAb approved specifically for cancer therapy. In the early years following its release, a relationship between cytokine release syndrome in patients and high lymphocyte counts was observed after treatment with the mAb. Patients with lymphocyte counts greater than 50×10^9/L experienced a severe cytokine release syndrome shown by peaks in release of TNF and IL-6 90 min after infusion and accompanied by fever, chills, nausea, vomiting, hypotension, dyspnea, an increase in liver enzymes, and prolongation of the prothrombin time. When used to treat B cell

cancers, the frequency and severity of first-dose reactions to rituximab were shown to be dependent on the initial number of circulating tumor cells—patients with counts exceeding 50×10^9/L experienced more adverse reactions than patients with lesser numbers of peripheral tumor cells.

Recently published results of a survey of rituximab hypersensitivity reactions in patients at Massachusetts General Hospital between 2006 and 2010 showed that immediate hypersensitivity reactions to rituximab occurred in 8.8 % (79 of 901) of patients treated with the mAb and premedications. Approximately three-quarters of the patients developed symptoms after the first infusion and 46 % of moderate or severe reactions occurred on subsequent infusions. Severity of reactions correlated with the risk of a recurrent adverse response—all patients with severe, and 56 % of those with moderate reactions, but only 36 % of patients with mild reactions, experienced a recurrence. Interestingly, there was an increased risk of a moderate or severe hypersensitivity reaction in those with advanced disease. Waldenstrom's macroglobulinemia accounted for 10 % of all reactions while representing only 1 % of patients treated with rituximab. In another retrospective analysis of reactions occurring over a 2-year period following infusion of rituximab in the treatment of multiple sclerosis, 25.7 % of patients had mostly mild to moderate reactions, most often during the first infusion. Most patients completed the infusion and went on to subsequent infusions without reactions. In common with a number of other surveys on rituximab use, it was concluded that infusion-related reactions to the therapeutic agent in patients with multiple sclerosis are common, premedication with drugs including corticosteroids dramatically reduces the incidence of reactions, and reactions that do occur can be effectively managed by treatment with H_1 and H_2 antihistamines and infusion rate adjustments.

As well as infusion-related reactions and reactions related to the number of circulating target cells, a number of post-infusion hypersensitivity or hypersensitivity-like reactions occur to rituximab. These reactions include serum sickness, vasculitis, various cutaneous manifestations, interstitial pneumonitis, and acute respiratory distress syndrome. Respiratory events such as cough, dyspnea, and bronchospasm are fairly common adverse reactions, but serious reactions such as fatal pulmonary fibrosis also occur. A review of 62 cases of rituximab-induced severe respiratory reactions revealed that 74 % suffered from interstitial pneumonitis and other respiratory problems including pulmonary fibrosis, bronchiolitis obliterans, organizing pneumonia, hypersensitivity pneumonia, and acute respiratory distress syndrome. Most patients were elderly but two pediatric patients, both with refractory nephrotic syndrome, were affected. Such findings have led to the suggestion that rituximab should never be administered to patients suffering from lung diseases such as pneumonia, pleural effusion, and atelectasis. Other mAbs including infliximab, gemtuzumab, OKT3, and a mAb-ozogamacin conjugate have also been implicated in cases of acute respiratory distress syndrome which is believed to be mediated by pro-inflammatory cytokines. Cutaneous side effects of rituximab are frequent, generally occur from 1 to 13 weeks after exposure, and are not usually serious but Stevens–Johnson syndrome, toxic epidermal necrolysis, lichenoid dermatitis, and vesiculobullous dermatitis have been described.

11.1.3.6 The Next Generation of Monoclonal Antibodies

Until now, FDA-approved mAbs (Table 11.1) are usually full-length antibodies, making them large molecular weight (~150 kDa) therapeutic agents with sometimes poor tissue penetration, especially for solid tumors. As understanding of the biological mechanisms in different diseases increases and genetic engineering technology advances, attention is turning to improving the performance and efficiency of mAbs in terms of increased selectivity, improved pharmacokinetics, higher binding affinities, more efficient cytotoxicity, better tissue penetration, and increased half-life in serum. More than 50 % of mAbs in phase I clinical trials and about 40 % of those in phases II and III are now modified antibodies such as antibody–drug conjugates, bispecific antibodies, antibodies with modified Fc

functions, and antibody fragments/domains. Employment of two mAbs may sometimes produce a better therapeutic outcome than one antibody alone, for example, the combination of cetuximab with specificity for EGFR and bevacizumab which recognizes VEGF, in the treatment of metastatic colorectal cancer. A more logical and efficient alternative to the use of more than one mAb is the creation of an antibody with specificities for two different targets, that is, bispecific antibodies. By simultaneously binding to two different complementary sites on a cell, bispecific antibodies may enhance binding selectivity, avidity, and tissue distribution, expand the mAb's disease indications, and increase antibody load on target cells. Nonspecific toxicity of chemotherapy is a major limitation of much of today's drug therapy for cancers (see chapter 13). Antibodies conjugated to carefully selected drugs or toxins delivered to specific tumor sites have the potential to reduce systemic toxicity and there is little doubt that such mAb drug conjugates will be increasingly developed and applied in future mAb immunotherapies. One recent imaginative approach in applying antibody–drug conjugates for cancer therapy is based on the occurrence of angiogenesis in virtually all types of aggressive cancers but the rarity of the process in healthy adults. Early experiments have demonstrated that strong antitumor activity in vivo can be achieved with vascular targeting of an antibody–drug conjugate that does not require antibody internalization. Fc engineered mAbs can be utilized for improved antibody-dependent cellular and complement-dependent cytotoxicities via their Fc regions and antibody fragments and single domain antibodies offer the opportunity to retain or enhance the binding properties of full-length antibodies by varying their size, valency, and pharmacokinetic profiles. Another potential improvement offered by antibody fragments is improved tissue penetration although this may be offset by a shorter serum half-life. Approaches already employed to overcome this include the chemical addition of polyethylene glycol (PEG) or so-called PEGylation to increase the size of fragments. This strategy was applied to certolizumab, an anti-TNF Fab fragment.

What effects these developments will have on the incidences and nature of adverse, and in particular allergic, reactions to therapeutic mAbs remains to be seen, but it is certain that the number and variety of genetically engineered mAbs and modified mAbs will greatly increase in the immediate future. We might expect that many reactions already encountered with the existing approved antibodies will still be seen, but we should not be surprised if some new, unanticipated adverse reactions emerge as newer generation agents showing some physical, chemical, and biological differences become established and increasingly used. However, as summarized above, the carefully planned strategies already being applied to modify and thereby improve the performance and tolerability of next generation mAbs (e.g., smaller size and intrabodies) may also carry with them a decreased capacity to provoke adverse responses in patients. In any case, the continuing elucidation of cellular pathways in an expanding range of diseases coupled with a deeper understanding of the myriad immunological and inflammatory processes and the advancing bioengineering expertise should give hope that such intellectual and technical ingenuity could also be applied to minimizing the detrimental effects of these precisely engineered therapeutic agents. Efforts expended to make mAbs more immunologically acceptable to humans have been impressive; equal efforts directed at minimizing adverse reactions might prove just as successful.

11.2 Etanercept

Like the mAbs infliximab, certolizumab, adalimumab, and golimumab, etanercept is specific for, and binds to, TNF, thus making it useful for the treatment of some autoimmune diseases including rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, and Crohn's disease. TNF receptors are of two types, those on nucleated cells and soluble receptors that circulate and deactivate the cytokine. Etanercept is a recombinant, engineered, fully human dimeric fusion protein of molecular weight 150 kDa, made up of

the extracellular ligand-binding portion of human 75 kDa TNF receptor (TNFR) linked to an Fc portion of human IgG1. The Fc portion contains the CH2 and CH3 domains and the hinge region but not the CH1 domain. In this form, the protein acts as a "decoy" receptor for TNF, mimicking the natural soluble receptor and improving on it by possessing a longer half-life (115 h; compare infliximab half-life 210 h). Both infliximab and etanercept are sometimes used for patients with rheumatoid arthritis when other disease-modifying antirheumatic drugs have failed. The most common side effects reported for etanercept include mild reactions at the injection site, infections (mainly upper respiratory), and sinusitis. U.S. FDA data on etanercept adverse events lists, in order of frequency, infections, followed by dermatologic, neurologic, musculoskeletal, pulmonary, cardiac, and vascular effects. Both etanercept and infliximab have been associated with cutaneous vasculitis in which symptoms coincided with their administration and symptom resolution coincided with treatment withdrawal. Lesions associated with etanercept-induced autoimmune skin reactions include purpuric papules and erythematous papules and nodules on the trunk and extremities. In one reported case of cutaneous vasculitis associated with both etanercept and infliximab, vasculitis provoked by etanercept in a patient with Crohn's disease was found to worsen significantly after therapy was switched to infliximab.

11.3 Interferons

Interferons (IFNs) are an important class of broad spectrum antiviral cytokines found in higher animals, reptiles, fish, and birds. IFNs make up a large, still growing list of proteins, seven of which occur in humans. These are further divided into three classes types I, II, and III. Two important type I IFNs in human are IFNα and IFNγ. There are at least 14 different alpha IFNs. These are produced by leukocytes and dendritic cells and are part of an innate immune response with antiviral action. IFNβ, produced by fibroblasts and probably many other cells, is used in the treatment of multiple sclerosis. It inhibits viral replication and is the product of a single gene. Two synthetic IFNs, pegylated IFNα-2a and pegylated IFNα-2b, have found application as antiviral agents in the treatment of hepatitis C virus. IFNα, now usually given in pegylated form, in combination with ribavirin (1-β-D-ribofuranosyl-1*H*-1,2,4,-triazole-3-carboxyamide) is the mainstay of treatment of hepatitis C infection. Pegylation enhances the half-life of the IFN by covalent attachment of polyethylene glycol (PEG) polymer chains to the molecule, reducing both antigenicity and immunogenicity. Adverse reactions to IFNα include flu-like symptoms, localized inflammation, and a wide range of cutaneous reactions including maculopapular rash, pruritus, urticaria, angioedema, pemphigus, vasculitis (and systemic), fixed drug eruption, and lichen planus. Autoimmune disorders thrombocytopenia and hemolytic anema also occur. Cytokine release syndrome probably accounts for many reactions with flu-like symptoms. Recombinant IFNβ has been implicated in anaphylactic shock, indicating that IgE-mediated reactions are probably involved in a few immediate reactions. Of 51 patients given pegylated IFNα-2b-ribavirin, 10 were found to have experienced serious adverse drug reactions after 1 month and adverse reactions were the main reason for discontinuing the therapy in eight patients (15.7 %). The most common reaction to this therapeutic agent is localized skin lesions at the injection site, but vesicle erythematous eruptions and autosensitization dermatitis away from the injection site have been described.

11.4 Interleukin 2

The cytokine interleukin 2 (IL-2) is necessary for T cell growth, proliferation, and differentiation. An FDA-approved recombinant human IL-2 is used clinically as Proleukin® (Prometheus Laboratories) for the treatment of metastatic melanoma and renal cell carcinoma. Erythema during IL-2 immunotherapy is common and was well described in a French study nearly 20 years ago with a report on generalized erythema followed by desquamation in 12 patients treated with the cytokine for renal cancer. Urticaria in eight renal cell cancer patients after the end of IL-2 therapy has also been reported. Skin tests with IL-2 on

two of the patients proved negative. Cutaneous side effects in metastatic melanoma patients treated with IL-2 were observed in 53 of 78 treatment cycles of 25 patients (72 %); 53 were mild reactions with a burning pruriginous erythema, and three were severe with urticaria, necrotic lesions, and blisters. Regression proved constant without sequelae. Other observed cutaneous reactions to IL-2 include injection site reactions, exfoliative dermatitis, angioedema, reactivation of eczema, vasculitis, pemphigus, and exacerbation of psoriasis. There appear to be no reports of anaphylaxis following IL-2 immunotherapy.

11.5 Denileukin Diftitox and Aflibercept

Diphtheria toxin is a single polypeptide chain of 535 amino acids. For use as a targeted toxin, it has been modified by deleting the 147 amino acid residue receptor-(cell-) binding domain to produce a protein of 388 amino acids commonly referred to as DT_{388} or DAB_{389}. This remaining protein consists of the adenosine diphosphate (ADP)-ribosyltransferase and membrane translocating domains of native diphtheria toxin. Replacing the receptor-binding domain of the native toxin by the sequences encoding the IL-2 gene produced the recombinant fusion toxin designated DAB_{389}IL-2 or denileukin diftitox (Ontak®, Eisai) which was approved by the FDA in 1999 for the treatment of cutaneous T cell lymphoma. Bound to the IL-2 receptor, the fusion toxin undergoes endocytosis and is proteolytically cleaved liberating the modified toxin and causing ADP-ribosyltransferase-mediated inhibition of protein synthesis. In phase I and III studies on DAB_{389}IL-2, infusion-related acute hypersensitivity reactions occurred in 70 % of patients and vascular leakage occurred in 27 % forcing 29 % of patients to discontinue therapy. There were no correlations between these reactions and the dose or the half-life of the fusion toxin. Since only the antihistamine diphenhydramine and acetaminophen were used for premedication in these studies, the steroids prednisone and dexamethasone were examined to see if they improved tolerability of DAB_{389}IL-2. Results showed that the incidence of acute infusion reactions decreased significantly with only three patients experiencing reactions and only two patients developing vascular leakage. Overall, a significant improvement of 60 % occurred when premedication with steroids was employed. Cutaneous reactions to denileukin diftitox include injection site reaction, erythema, and pruritus, and there has been one fatal case of toxic epidermal necrolysis.

Another recombinant fusion protein, *aflibercept* (Zaltrap®), used to treat colorectal cancer and macular degeneration, is a chimera of the Fc piece of IgG1 and the extracellular ligand-binding domains of human vascular endothelial growth factor receptors VEGFR1 and VEGFR2. Adverse/hypersensitivity reactions to the drug include cytopenias, hemorrhage, thromboembolism, GI perforation, acral erythema and stomatitis.

11.6 Anakinra

Interleukin-1 (IL-1) is a cytokine produced in response to inflammatory stimuli in conditions such as rhinitis, rheumatoid arthritis, and other immunological reactions. The IL-1 receptor (IL-1R) exists in both membrane-bound and soluble forms and is expressed on many organs and tissues. Anakinra, a specific receptor antagonist for IL-1, is a 153 amino acid non-glycosylated, molecular weight 17.258 kDa recombinant protein prepared in *Escherichia coli*. It differs from natural IL-1R by the addition of a single methionine added to the amino terminal end. The recombinant receptor antagonist competes with IL-1, blocking its access to its complementary receptor, thus making it a useful agent in the treatment of some inflammatory conditions such as rheumatoid arthritis where it acts as a biological response modifier rather than a disease-modifying antirheumatic drug. The protein has a half-life of 4–6 h and peak plasma concentrations occur 3–7 h after subcutaneous injection. In an examination of the safety profile of anakinra, over 1,300 patients were initially studied in a double blind trial comparing the drug (100 mg/day) with placebo before proceeding to open-label treatment for up to 3 years. All adverse events were similar in the anakinra and placebo groups and for each of the years of anakinra treatment. Injection

site reactions were the most frequent adverse event (122 events/100 patient years) and, overall, it was concluded that anakinra is safe and well tolerated for up to 3 years of continuous use. Cutaneous reactions are usually at the injection site and usually well tolerated. Skin biopsy specimens from rheumatoid arthritis patients treated with anakinra and with well-defined erythema and edema at the injection sites showed marked dermal edema, an increased number of mast cells, and a lichenoid infiltrate of mainly lymphocytes together with eosinophils and CD68 macrophages. In some cases, cutaneous reactions were associated with systemic involvement. The observed skin reactions were said to resemble reactions seen in patients receiving chemotherapy and colony-stimulating factors. A cutaneous reaction in one patient was shown to be mediated by specific IgE antibodies. After several injections of anakinra, an adult female patient began to experience erythema and pruritus at the injection site within 15 min after each injection. No systemic symptoms occurred, but 1 day later swelling, erythema, and pruritus were apparent and these persisted for a further day or two. Prick and intradermal tests together with an ELISA for specific IgE antibodies to anakinra were undertaken. Prick tests with concentrations of 500 and 2,500 μg/ml were negative, but intradermal testing with 125 μg proved positive. The reaction was still positive after 48 h. Five normal control subjects had no reaction to the same dose of the drug. The specific IgE test was also positive and this was confirmed by inhibition experiments with free anakinra.

11.7 Anti-thymocyte Globulin

Indicated and approved for the management of allograft rejection in renal transplant patients, anti-thymocyte globulin preparations are purified immune globulins (primarily IgG) from horses or rabbits immunized with human thymus lymphocytes. The resultant globulin preparations contain cytotoxic antibodies to human T lymphocytes which function as an immunosuppressive agent. As well as its use for the treatment of renal transplant rejection, anti-thymocyte globulin may be administered as an adjunct to other immunosuppressive therapy to delay rejection. Two preparations are available, Thymoglobulin® (Genzyme), obtained by immunizing rabbits, and Atgam® (Pfizer), from hyperimmune horse serum. These two preparations are contraindicated in patients with a history of allergy and anaphylaxis to rabbits or horses, respectively. Serious immune-mediated reactions have been reported, including anaphylaxis, severe cytokine release syndrome, and severe acute infusion-associated reactions. The last named may occur as early as the first or second infusion and serum sickness with fever, rash, arthralgia, and myalgia may appear 5–15 days after the initiation of therapy. As a precaution against the possibility of anaphylaxis, it is recommended that before the first infusion, patients should be tested intradermally with the diluted globulin preparation (for example, with ~5 ug of horse globulins). The most frequently seen adverse reactions, that is, seen in more than 25 % of patients, include fever, chills, headache, nausea, diarrhea, abdominal pain, peripheral edema, hypertension, thrombocytopenia, infection, dyspnea, and tachycardia. Cutaneous reactions seen include urticaria, morbilliform eruptions, and acral erythematous eruptions preceding rash. Discontinuation of treatment with anti-thymocyte globulin is recommended upon the appearance of systemic reactions such as anaphylaxis, generalized rash, tachycardia, dyspnea, hypotension, severe thrombocytopenia, or severe leukopenia.

11.8 Epoetins

Human erythropoietin (or erthropoetin) (EPO), also called hematopoietin (or hemopoietin), is a glycoprotein hormone of 165 amino acids MW 34 kDa that controls erythropoiesis (red blood cell production). Recombinant human EPO, introduced in 1986, is available as epoetins alfa, beta, delta, and omega each differing from the endogenous hormone, and from each other, by the individual sugar and sialic acid residues present. Epoetins are administered for renal and non-renal anemias and, despite being given to a large number of patients over a period of 25 years, the preparations have proved poorly immunogenic and few side effects have been observed. In 2001 the FDA and European

Medicines Agency approved a new epoetin, darbepoetin alfa, for treatment of anemia due to renal failure and in patients undergoing immunotherapy. Darbepoetin alfa is a recombinant epoetin molecule containing an extra two *N*-linked oligosaccharide chains introduced to give greater stability and thus allow less frequent administrations. In recent years, there appears to have been an increase in the number of patients developing neutralizing anti-EPO antibodies during therapy and there are now in excess of 250 known cases of pure red cell aplasia. There are also reports of anaphylaxis, rashes, urticaria, and angioedema to the recombinant hormone and injection site reactions are well known. Cutaneous reactions at the sites of former subcutaneous injections of epoetins following the IV injection of different epoetins proved to be the signs of an allergic skin and systemic reaction in a patient with pure red cell aplasia and anti-EPO antibodies. After switching the patient from epoetin alfa to first epoetin beta and then darbepoetin alfa, a systemic anaphylactic/anaphylactoid response occurred and anti-EPO antibodies cross-reactive with epoetin beta and darbepoetin alfa were detected in the patient's serum. This case illustrates that continuation of epoetin therapy in patients with anti-EPO antibodies may carry the risk of a serious systemic (anaphylactic or anaphylactoid) reaction and that skin reactions at the injection site may be the first sign of sensitization which precedes the development of anemia. In three other rare cases, anaphylaxis and serum IgE antibodies, and a generalized eczematous reaction to recombinant EPO, were reported in the presence of negative skin tests to the glycoprotein and acute exanthematous pustulosis was diagnosed after epoetin alfa was replaced by darbepoetin alfa. In an unexpected EPO allergy "false-alarm," the nonionic surface active agent polysorbate 80, and not Eprex® (Janssen) containing epoetin alfa, was shown to be responsible for a case involving generalized pruritus, erythema, and angioedema.

11.9 Human Insulin

Adverse reactions to insulin were not uncommon in the past when the administered preparations were from bovine and porcine sources. The introduction of human recombinant insulin reduced the incidence of reactions, but allergic reactions are still occasionally seen and insulin allergy is now reported to be less than 1 % of diabetic patients treated with insulin. As early as 1982, IgE antibodies to human recombinant insulin that cross-reacted with bovine and porcine insulins were demonstrated in two diabetic patients previously untreated with insulin. Although these patients did not develop any of the clinical manifestations of insulin allergy, large local reactions in association with IgE to human insulin were later seen in a patient within 12 days of insulin initiation therapy with the human recombinant product. The patient showed similar cutaneous reactivity with bovine and porcine insulins despite never having received those preparations, and the insulin-reactive IgE antibodies cross-reacted in vitro with the heterologous proteins. This strongly indicated that common, or markedly similar, antigenic determinants are present on the insulins from all three species. Subsequent experience has confirmed these findings. Skin testing as well as IgE antibody measurements have clearly demonstrated immediate type I reactions to recombinant human insulin and to bovine and porcine insulins in patients never previously given the heterologous insulins. In the investigation of an anaphylactic reaction to human recombinant insulin, employment of skin testing and the Novo Insulin Allergy Kit (Novo Nordisk A/S, Bagsvaerd, Denmark) showed positive intracutaneous tests to 1–100 dilutions of human and porcine insulins and to the genetically engineered recombinant insulin analog, insulin lispro. Once again, the patient had never been treated with porcine insulin in the past. A number of different models have been applied in attempts to desensitize patients allergic to recombinant human insulin. In one successful attempt, insulin lispro was delivered as a continuous infusion via an insulin pump. The delivery and dosage schedule was: 0.7 IU/h for 2–8 h; 0.3 IU/h for 8–13 h; 0.6 IU/h for 13–18 h; 0.8 IU/h for 18–21 h; and 0.6 IU/h for 21–22 h, plus an additional bolus of 6 IU before breakfast, 5 IU before lunch, and 6 IU before dinner. Following this procedure, the allergic reaction did not reoccur. Although the patient remained clinically asymptomatic, the skin prick test to insulin remained positive 3 months later.

Summary

- MAbs were first produced by mouse hybridoma cells prepared by fusing spleen cells from an immunized mouse with mouse myeloma cells. The hybridoma cells retain the capacity to make specific antibody while the myeloma cells impart the capacity of the cells to grow indefinitely in culture, continuously secreting antibody.
- Because mouse antigens rapidly induce an immune response in humans, methods have been developed to humanize mAbs. One approach involves the production of chimeric antibodies. Other methods now produce fully humanized antibodies.
- MAbs of murine origin are designated by the stem *-omab*; chimeric antibodies in which the variable region is spliced into a human constant region are given the *-ximab* stem; humanized antibodies with the murine hypervariable regions spliced into a human antibody have the *-zumab* stem and antibodies with a complete human sequence are given the *-mumab* or *-umab* suffix.
- Currently, ~28–30 mAbs are approved, or under consideration for approval, as specific therapies in the USA or European Union, although about 350 new mAbs for therapeutic application in humans are in the commercial pipeline.
- So far, the number of target antigens for the mAbs is surprisingly small with more than one of the approved antibodies specific for TNF, HER2, CD20, EGFR, or VEGF. Other specificities include EpCAM, glycoprotein IIb/IIIa, CD30, CD52, C5, α-4 integrin, IgE, IL-6R, BLys, IL-1β, and RANK-L.
- Initial infusion reaction to some mAbs, for example, rituximab, may provoke tumor lysis syndrome, cytokine release syndrome, and systemic inflammatory response syndrome.
- Omalizumab, a humanized IgG1κ mAb with specificity for human IgE antibodies, is approved for the treatment of severe allergic asthma. It binds to free, circulating IgE antibodies and membrane-bound IgE molecules on some cells such as B lymphocytes expressing the antibody, but it does not bind to IgE already bound to mast cells. The incidence of anaphylaxis to the mAb is about 0.2 %.
- Some patients receiving cetuximab experienced severe immediate hypersensitivity reactions. The antibodies involved were found to be IgE, specific for α-D-galactose-(1–3)-β-d-galactose and reactive with this disaccharide present on the Fab portion of the chimeric antibody at asparagine 88 of the heavy chains. Some cases of anaphylaxis to the mAb appear to be associated with tick bites and consumption of red meat.
- Systemic and cutaneous reactions have been reported following administration of infliximab. These include anaphylaxis, serum sickness, maculopapular rashes, urticaria, psoriasis, flare-up of atopic dermatitis, and leukocytoclastic vasculitis. The overall incidence of infusion reactions in one study was 6.1 %. Mild, moderate, and severe reactions occurred in 3.1, 1.2, and 1 % of infliximab infusions, respectively.
- Patients with lymphocyte counts greater than 50×10^9/L experienced a severe cytokine release syndrome shown by peaks in release of TNF and IL-6 90 min after infusion with rituximab.
- A number of post-infusion hypersensitivity or hypersensitivity-like reactions occur to rituximab. These reactions include serum sickness, vasculitis, various cutaneous manifestations, interstitial pneumonitis, and acute respiratory distress syndrome.
- As genetic engineering technology advances, attention is turning to improving the performance and efficiency of mAbs in terms of increased selectivity, improved pharmacokinetics, higher binding affinities, more efficient cytotoxicity, better tissue penetration, and increased half-life in serum.
- Etanercept is a recombinant, engineered, fully human dimeric fusion protein of molecular weight 150 kDa made up of the extracellular ligand-binding portion of human 75 kDa TNF receptor linked to an Fc portion of human IgG1. U.S. FDA data on etanercept adverse events lists, in order of frequency, infections, followed by dermatologic, neurologic, musculoskeletal, pulmonary, cardiac, and vascular effects.
- Two synthetic IFNs, pegylated IFNα-2a and pegylated IFNα-2b, have found application as antiviral agents in the treatment of hepatitis C virus.

- Adverse reactions to IFNα include 'flu'-like symptoms, a wide range of cutaneous reactions, autoimmune thrombocytopenia, and hemolytic anemia.
- Reactions to IL-2 immunotherapy include erythema, urticaria, and a variety of other cutaneous reactions. There appears to be no report of anaphylaxis.
- Replacing the receptor-binding domain of the diphtheria toxin by the sequences encoding the IL-2 gene produced the recombinant fusion toxin designated DAB_{389}IL-2 or denileukin diftitox. Cutaneous reactions to denileukin diftitox include injection site reaction, erythema, and pruritus. There has been one fatal case of toxic epidermal necrolysis.
- Aflibercept, used to treat metastatic colorectal cancer and wet macular degeneration, is a fusion protein of the Fc piece of IgG1 and the extracellular ligand-binding domains of human vascular endothelial growth factor receptors VEGFR1 and VEGFR2. It acts as a soluble decoy VEGF receptor and angiogenesis inhibitor. Common adverse reactions include cytopenias, hemorrhage, proteinuria, hypertension, acral erythema, hyperpigmentation, and stomatitis.
- Anakinra, a specific receptor antagonist for IL-1, is a 153 amino acid non-glycosylated, molecular weight 17.258 kDa recombinant protein prepared in *E. coli*. Cutaneous reactions following anakinra medication are usually at the injection site and usually well tolerated. In some cases, cutaneous reactions are associated with systemic involvement.
- Serious immune-mediated reactions to antithymocyte globulin include anaphylaxis, severe cytokine release syndrome, and severe acute infusion-associated reactions. Serum sickness with fever, rash, arthralgia, and myalgia may appear 5–15 days after the initiation of therapy. Cutaneous reactions seen include urticaria, morbilliform eruptions, and acral erythematous eruptions preceding rash.
- Reactions to epoetins are rare, but anaphylaxis to the recombinant forms of the hormone EPO have been reported, one in particular after injection site reactions in a patient with pure red cell aplasia.
- Allergic reactions to recombinant human insulin with cross-reactivity to bovine and porcine insulins occasionally occur.

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Corticosteroids

12

Abstract

Despite their anti-inflammatory and immunosuppressive properties, corticosteroids (CSs) occasionally provoke immediate type I and delayed type IV allergic reactions. Frequency of reactions after topical application is 0.2–5.98 %; for systemic reactions, the incidence is 0.1–0.3 %. The reaction of steroid glyoxals with arginine is important for allergenicity of CSs. Allergic contact eczema is the most common delayed hypersensitivity reaction to CSs. Type IV reactions are mainly diagnosed by patch testing with tixocortol pivalate, budesonide, and hydrocortisone 17-buyrate being the principal drugs employed for testing. A combination of tixocortol pivalate and budesonide detects over 91.3 % of allergic patients. CSs have been classified into four structural groups A, B, C, and D on the basis of clinical cross-reactivity patterns obtained from patch test results. Recently, a new classification divides the CSs into three groups (1) Those that produce most of the allergic reactions—CSs that are non-methylated and usually nonhalogenated. (2) Halogenated molecules with a C16/C17 *cis*-ketal/diol structure. (3) CSs that produce allergy rarely—halogenated and C16-methylated CSs. Anti-inflammatory and immunosuppressive actions of CSs do not seem to affect immediate reactions. Skin testing, both prick and intracutaneous, together with challenge tests form the basis of the diagnosis of immediate systemic reactions to CSs.

12.1 Introduction, Incidence of Allergy, and Sensitization

Although it might seem counterintuitive that drugs with pronounced anti-inflammatory and immunosuppressive properties can provoke both immunological and inflammatory responses, corticosteroids (CSs) do occasionally induce immediate type I and delayed type IV allergic reactions. Type II cytotoxic and type III immune complex reactions do not appear to have been reported. Leaving aside their pharmacological activities, these drugs are so widely and heavily used, their relative rarity in eliciting reactions might be seen as unusual. In fact, when reactions

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_12, © Springer Science+Business Media, LLC 2013

to CSs do occur, there is the likelihood that they may be masked by the drugs' anti-inflammatory properties and diagnoses of allergic reactions may be missed. In cases of contact allergy, symptoms are often minor and not always obvious, and lesions are rarely alarming and generally chronic. Reports of incidences of allergy to CSs vary, sometimes markedly. For reactions after topical application, frequencies from 0.2 to 5.98 % are claimed. From 11,596 European patients surveyed over a period of 18.5 years, patch tests revealed 315 with at least one reaction to a CS, which is an incidence of 2.7 %. Eighty five percent of the 315 patients reacted to more than one CS. An overall incidence of 2.6 % was shown in a multicenter study from 10 European countries undertaken by the European Environmental and Contact Dermatitis Research Group. Results were variable, ranging from 0.4 to 0.6 % in Portugal and Spain to 6.4 % in Belgium. A retrospective study of patch testing at the Mayo Clinic, USA, of 1,188 patients over a 6 year period (2000–2005) revealed 10.7 % of patients reacted to at least one CS and 4.7 % reacted to multiple CSs. Tixocortol pivalate alone detected less than 50 % of the allergic patients.

Patients allergic to a CS often react to multiple allergens, but it remains unclear whether atopy is a risk factor for CS hypersensitivity. A history of atopy was found in 34 % of the 315 CS-allergic patients but overall, it is difficult to determine whether a higher incidence is due to increased susceptibility or greater exposure. Few reactions in asthmatics have been noted despite the extremely widespread use of inhaled CSs. Risk factors for systemic sensitivity do not seem to have been studied but some reports indicate a higher incidence in asthmatic subjects.

The extensive use of CSs is affected by all delivery routes—parenterally, via cutaneous conjunctival, nasal, oral and rectal mucosa, and the respiratory and gastrointestinal tracts, so there is ample opportunity for sensitization to these drugs to occur. The risk of sensitization via topical application is highest for patients with long-term skin conditions such as chronic eczema, hand and foot dermatitis, and stasis dermatitis suggesting the influence of skin barrier breakdown and involvement of antigen-presenting Langerhan's cells. A surprising finding in the study of the 315 European CS-allergic patients was the discovery that 15 of 22 sensitized by the inhalation of steroids were not themselves treated by CS aerosols—sensitization apparently resulted from exposure to CS used by others in their environment who they were taking care of. Sensitization by airborne exposure to budesonide in ophthalmic preparations was also found in 19 patients. Forty-five of the 315 patients (14 %) had previous exposure to systemic CSs.

For systemic reactions, the best estimate so far of the incidence of reactions is 0.1–0.3 %.

12.2 Corticosteroid Haptens: Metabolism and Degradation

It is currently believed that the allergenic structure of CSs hydroxylated or esterified at C21 (e.g., as occurs with hydrocortisone (Fig. 12.1a) and methylprednisolone succinates) are not the unaltered parent drugs but a metabolite formed within the skin when the CS degrades to a reactive glyoxal that binds to nucleophilic protein residues. Steroid glyoxals react with most of the amino acids but the reaction with arginine predominates and seems to be the most important since those CSs with the greatest capacity to bind to arginine are also the strongest allergens. The breakdown of the C21 esters is unaffected by the length of the carbon chain. It is not clear what mechanisms operate, if any, to form altered allergenic structures from CSs that are neither hydroxylated nor esterified but have a halogen atom at C21 (e.g., clorbetasol and halcinonide). Substituents on the gonane nucleus of CSs also affect the anti-inflammatory activity and the rate of degradation of the compounds. The oxygen at C11 enhances degradation whereas the β-methyl group at C16 and hydrogen at C17 stabilize the molecule. Halogens at C6 and C9 have been claimed to both stabilize and determine allergenic cross-reaction patterns of CSs.

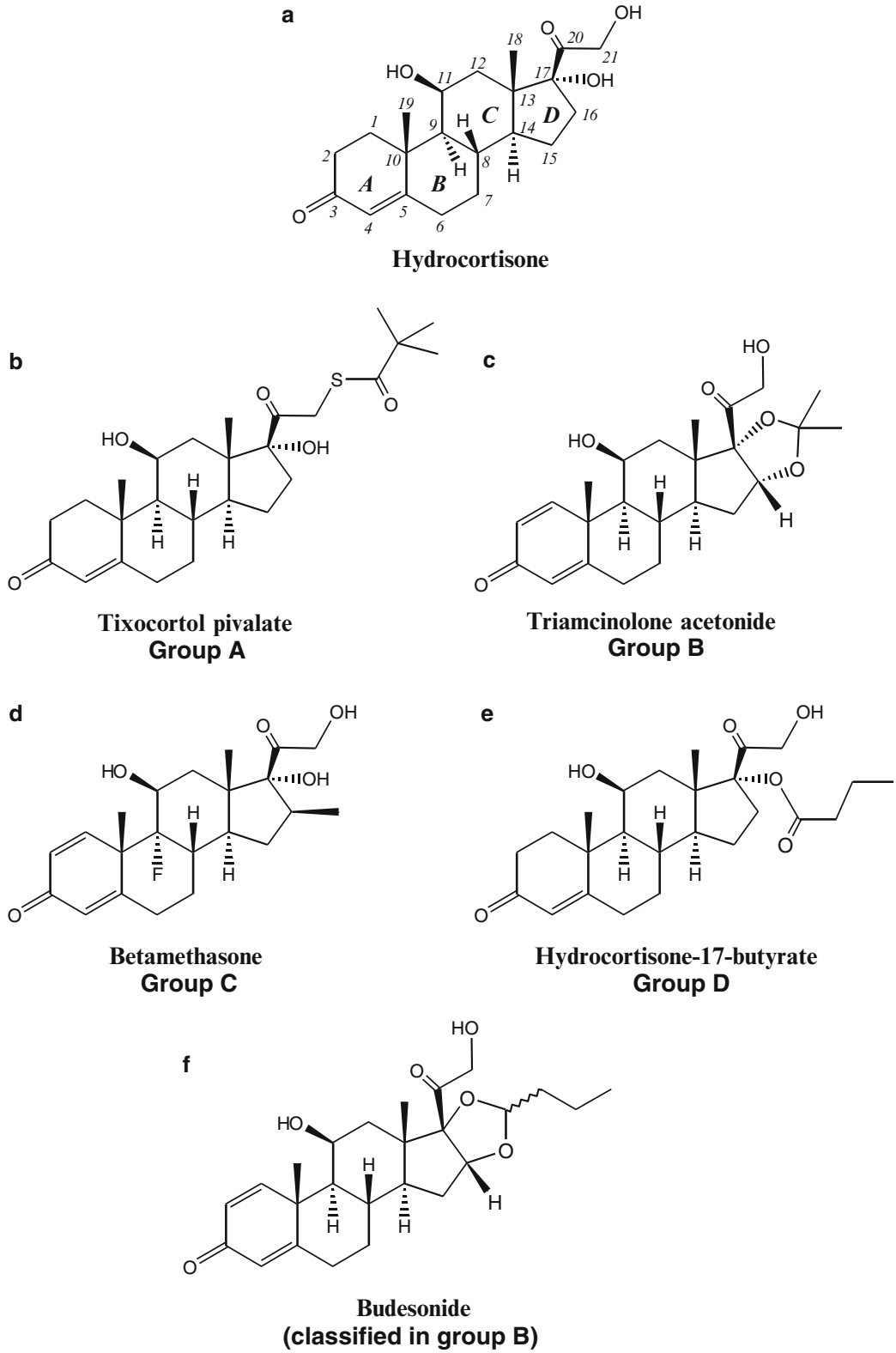

Fig. 12.1 Corticosteroids from the four structural groups **A**, **B**, **C,** and **D** classified on the basis of clinical cross-reactivity patterns obtained from patch test results. Drug examples from each of the four groups are shown. The basic structure of the CSs is the gonane or cyclopentaneperhydrophenanthrene nucleus of 17 carbons shown numbered on the hydrocortisone structure in **a**. Note that the *A–D* designation of the four different rings of the gonane nucleus should not be confused with the *A, B, C, D* classification of cross-reactivity

12.3 Delayed Reactions

12.3.1 Clinical Presentation

Reports of delayed hypersensitivity reactions to CSs include allergic contact dermatitis (Fig. 12.2), maculopapular rash, exanthematous, papulovesicular and flexural rashes, rash with or without bullae or purpura, acute generalized exanthematous pustulosis, and erythema multiforme. Allergic contact eczema is the most common delayed hypersensitivity reaction to CSs. Affected skin sites in order of frequency are hands, legs, face, feet, a generalized reaction, arms, eyes, trunk, and neck. Signs of a reaction are often minor, but if the time to reach a diagnosis has been lengthy, effects such as rosacea, perioral (Fig. 12.3) or perinasal dermatitis, and cutaneous atrophy may be seen. What may appear to be chronic eczema with "edge effects," that is, a reaction that is more pronounced at the periphery of the treated area, may in fact be allergic contact dermatitis induced by CSs. Reactions due to ocular use of CSs may manifest as facial edema, eczema, and conjunctivitis while those resulting from inhalant use include eczematous eruptions around the facial orifices with possible mucosal involvement. A small number of patients present with generalized eruptions and, of these, an occasional patient also has systemic symptoms such as hypotension and malaise. Following systemic CS administration, generalized maculopapular or eczematous eruptions may sometimes occur. Note that CS-sensitive diseases that are exacerbated by CS treatment, respond poorly or not at all, or reoccur soon after CS treatment is discontinued should be considered as a possible allergy to CSs.

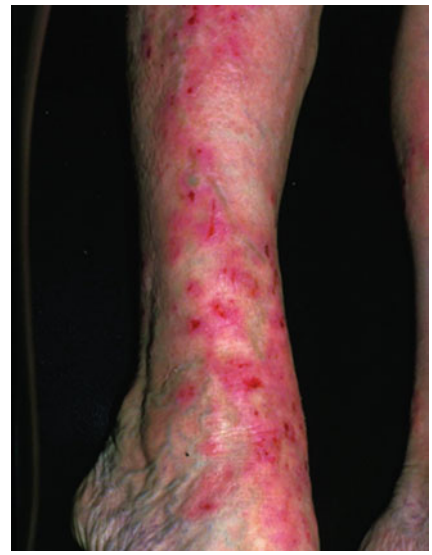

Fig. 12.2 Allergic contact dermatitis to hydrocortisone showing minor inflammation (Photograph courtesy of A. Goossens). From Brandão FM, in Johansen JD, Frosch PJ, Lepoittevin J-P, editors. Contact Dermatitis, 5th ed. Berlin: Springer-Verlag; 2011. Reprinted with permission from Springer Science + Business Media

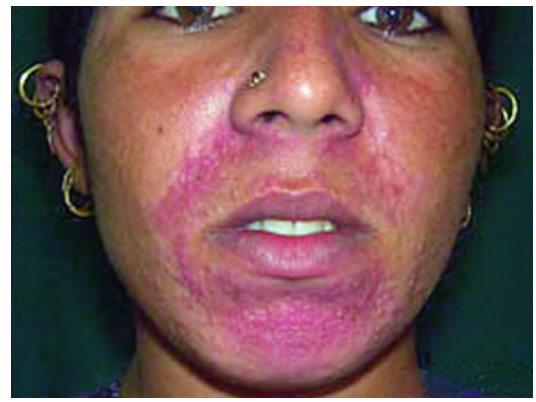

Fig. 12.3 Perioral dermatits. From Zaidi Z, Lanigan SW. Dermatology in Clinical Practice. London: Springer-Verlag; 2012. Reprinted with permission from Springer Science + Business Media

12.3.2 Diagnosis of Corticosteroid Hypersensitivity

12.3.2.1 Drugs for Patch Testing

Type IV reactions to CSs are mainly diagnosed by patch testing. Tixocortol pivalate, budesonide, and hydrocortisone 17-buyrate are the principal drugs employed for testing. An evaluation of the most useful CSs for the detection of CS contact allergy in 2,123 patients showed that 5.98 % of patients were allergic to one or more CSs; 4.5 % to tixocortol pivalate, 2.4 % to hydrocortisone butyrate, 2.2 % to budesonide, 0.52 % to each of betamethasone valerate and clobetasone butyrate,

and 0.38 % to clobetasol propionate. A combination of tixocortol pivalate and budesonide detected 91.3 % of CS-allergic patients suggesting that both should be included as standard test agents when patch testing for CS delayed hypersensitivity. Of the 315 patients positive to at least one CS in the aforementioned European study, 61 % reacted to budesonide, 43 % to tixocortol pivalate, 31 % to hydrocortisone 17-butyrate, and 22 % to prednisolone caproate. Importantly, positive tests to other CSs occurred with 91, 81, and 99 % of patients allergic to budesonide, tixocortol pivalate, and hydrocortisone 17-butyrate, respectively. Budesonide and tixocortol pivalate together detected 88.5 % of the CS-allergic patients and addition of hydrocortisone 17-butyrate increased the detection figure to 92.5 %. Of course, other CSs used by the patient should also be tested along with the appropriate vehicle controls. A mixture of the above three CSs detected less than 50 % of patients allergic to tixocortol pivalate (presumably because of masking of anti-inflammatory effects by the different drugs in the mixture) and therefore each drug should be tested separately.

Intradermal testing has been applied to the diagnosis of CS allergy but the atrophy-inducing effect of the drugs in this presentation form, particularly for suspensions, is a practical limitation. For CSs not known to induce atrophy, intradermal tests with solutions formulated at 30, 10, and 1 % in saline may be considered for particular cases, that is, for patients with a suggestive history of allergy but a negative patch test finding. In a comparison of patch testing with CSs in 1 % ethanol and intradermal tests with CSs suspended in physiological saline, tixocortol pivalate, and budesonide detected all patients allergic to hydrocortisone and budesonide, respectively, but patch tests with hydrocortisone 17-butyrate failed to detect 30 % of the positive reactions detected by intradermal testing. The study also demonstrated that use of ethanol as a vehicle for other CSs led to both false positive and false negative reactions. It was suggested that consideration should be given to the avoidance of hydrocortisone 17-butyrate testing in patients patch test positive to tixocortol pivalate and budesonide.

12.3.2.2 Concentrations of Drugs for Patch Testing

Care should be exercised by those seeking test concentrations of CSs in the literature since there is no universal agreement, and numerous authors have published different "optimum" figures. For example, in a study of contact allergy to CSs published in 2000, concentrations of 0.1 and 1 % in alcohol or petrolatum were recommended for budesonide and tixocortol pivalate, respectively. In a 2009 study with one author common to both publications, budesonide 0.01–0.02 % in petrolatum and 0.002 % in ethanol and 0.1 % tixocortol pivalate were said to detect most patients allergic to CSs. This is not surprising and is perhaps explained by the anti-inflammatory effect of too high a concentration of CS. This could be especially important in patients mounting weak reactions so it might be said that in such patients, an inverse relationship exists between positive patch tests and the concentrations of test agents. However, the thorough investigator should be prepared to try different concentrations of test materials, including higher concentrations, especially in patients with a negative test result but a clear clinical suspicion of allergy. Hydrocortisone 17-butyrate and most other CSs are tested at a concentration of 1 %.

12.3.2.3 Reading Patch Tests

Reading and interpretation of patch tests should always be undertaken by a well-trained and experienced person and the need for that requirement is even more obvious in the diagnosis of CS allergies. Because the anti-inflammatory effect of CSs can mask allergic reactions, too high a concentration of test agents may give false negative reactions and this has led to the recommendation of extended reading times to 6 or 7 days or even longer. The edge effect is a major factor to consider in reading patch test results for CSs. Manifesting as a clear area of no reaction surrounded by erythema, induration, and/or papulovesicles, this phenomenon is probably due to the anti-inflammatory effect of the CS in the center where the concentration is highest and the grading into lower concentrations as the drug diffuses outward where the anti-inflammatory effect

becomes less pronounced and the inflammatory reaction correspondingly more apparent. In later readings, this reaction pattern is less clear until the test site becomes entirely eczematous. Initial whitening of the skin test site due to vasoconstriction followed by vasodilation accompanied by erythema are other possible effects that can lead inexperienced readers of patch test results to interpret them as negative or positive reactions, respectively.

12.3.2.4 The Vehicle for Corticosteroids in Patch Testing

The amount of skin penetration of CSs used in testing is obviously important and this is to a large extent influenced by the vehicle employed. The drug needs to preferably be soluble, or at least well dispersed, and stable in the vehicle to ensure optimal presentation and availability. Vehicles most often used are ethanol, petrolatum, physiological saline, and sometimes dimethyl sulfoxide. For most CSs, ethanol is usually used but for hydrocortisone penetration is better with an equal mixture of ethanol and dimethyl sulfoxide as vehicle. Matura, in tests on CS-allergic patients with a large panel of CSs, did not detect significant differences between tixocortol pivalate in ethanol and petrolatum but budesonide and hydrocortisone 17-butyrate showed more positive reactions in ethanol than in petrolatum. Controls for patch testing should include each of the vehicles used. Positive patch test reactions have been reported for ethanol, ethanol/dimethyl sulfoxide, glutaraldehyde, and glyoxal. Preservatives and other agents added to CS formulations may also need to be ruled out as the elicitor of a positive reaction. Substances that are sometimes added in formulating topical CS preparations include neomycin, propylene glycol, benzyl alcohol, sorbic acid, wool alcohols, and many others. Where possible, these added substances should also be patch tested to ensure that they themselves do not provoke reactions.

12.3.2.5 Stability of Test Solutions

CSs have limited stability in ethanol; in one study the chromatographic purity of some CSs fell to 75–95 %. However, several investigations showed that fresh and stored solutions gave similar results in skin tests. In one comparative study of stabilities, budesonide, tixocortol pivalate, and hydrocortisone 17-butyrate in ethanol and petrolatum were kept at room temperature, refrigerated and deep frozen, and checked for stability by high performance liquid chromatography over a 1 year period. The ethanolic and petrolatum preparations of bunesonide and tixocortol pivalate remained stable for the entire storage period irrespective of the storage conditions. Hydrocortisone 17-butyrate 1 % in ethanol was also stable for the same period but was stable for only 3 months at room temperature. It has been suggested that retention of skin test reactivity after storage may be due to the breakdown products which are, in fact, the allergens rather than the parent compounds. As this subject remains sparingly investigated at best, it seems prudent to prepare fresh solutions of CS test agents at least every 4, or perhaps 6, weeks.

12.3.2.6 Other Diagnostic Tests

In the early 1990s, Wilkinson in England showed that a hydrocortisone–albumin complex could induce a proliferative response in peripheral blood mononuclear cells from patients allergic to the CS. In the same period, Lauerma and colleagues in Finland demonstrated flare-up in skin reactions after oral hydrocortisone in patients with contact allergy to the drug and proliferative responses to corticosteroids in T lymphocytes from patients with CS contact hypersensitivity. However, cell proliferation only occurred when epidermal Langerhans' cells were present and not when peripheral blood monocytes were present as antigen-presenting cells. The lymphocyte transformation test, discussed in Sect. 4.7.1, is said to have a sensitivity of 60–70 %, but there are a number of practical issues that make the test problematic for many clinical departments and even research laboratories. More recently, two cases of prednisolone-induced acute generalized exanthematous pustulosis were confirmed by positive skin and lymphocyte transformation tests to prednisolone and structurally related CSs. Other techniques like ELISPOT assays for cytokines such as IFN-γ (Sect. 4.7.3.2) appear to be potentially promising techniques to aid the diagnosis of delayed reactions to CSs.

12.3.3 Structure–Activity Relationships of Corticosteroid Cross-Reactions

The basic structure of the CSs is the gonane or cyclopentaneperhydrophenanthrene nucleus of 17 carbons. The CSs contain an extra two (as in testosterone) or four carbon atoms and some in the latter group, for example, cortisol or hydrocortisone, have a hydroxyl group at position 17 (Fig. 12.1a). In one of the few comprehensive structure–activity analyses undertaken in the field of delayed drug hypersensitivities, CSs were classified into four structural groups A, B, C, and D on the basis of clinical cross-reactivity patterns obtained from patch test results on 19 patients and from the literature. Drug examples corresponding to each group are: Group A, tixocortol pivalate; group B, triamcinolone acetonide; group C, betamethasone; group D, hydrocortisone-17-butyrate (Fig. 12.1b–e). The *D* ring was identified as the structural feature that distinguished the different groups, and it was suggested that allergic reactions occurred more often to CSs of the same group. (Note that the *A* to *D* designation of the four different rings of the gonane nucleus should not be confused with the A, B, C, D classification of cross-reactivity.)

Subsequently, this classification was supported by conformational analysis and statistical calculations on some CSs which were interpreted as showing that within each group A, B, and D, structures were homogeneous in volume, shape, and charge distribution. The situation with group C remained unclear. The findings were said to explain why cross-reactions between CSs from different groups are unlikely. Clinical cross-reactivity between budesonide (Fig. 12.1f), which is classified in group B, and some CSs in group D was also explained by conformational considerations. The clinical cross-reaction exhibited by this CS and group D compounds was said to be due to the *S* diastereoisomer of budesonide while cross-reactions with group B are said to arise from both the *R* and *S* forms. In terms of the structures recognized, budesonide, as an equal mixture of *R* and *S* diastereoisomers, is thought to cross-react with group B molecules via the acetonide group (bridged C16–C17). Cross-reaction with group D esters is thought to be due to the hydrophobic cavity present in the S isomer. This resemblance of different isomeric forms of budesonide to both the acetonide structure and C17 esters appears to explain why budesonide is such a useful patch test screening agent. About 90 % or more of individuals with a positive patch test reaction to budesonide are allergic to other CSs. Despite the apparent utility of the four category CS structure–activity classification, cross-reactions between different groups do occur, particularly between groups A and D, and consequently alternative explanations have been advanced. Substitutions of halogen atoms at carbons 6 and 9 on the B ring has been suggested as a basis of cross-reactivity between CSs but while this seems to hold for groups A, C, and D and positive reactions to fluorinated derivatives occur less frequently, there seems to be no difference between halogenated and nonhalogenated derivatives in group B. In a modification of the C6/C9 cross-reaction hypothesis, a second principal immune recognition site, viz. C16/C17 was also suggested. It seems therefore that all participants in the study of CS cross-reactions appear to agree on the importance of C16/17 substitutions on the *D* ring of the steroid backbone. However, in what is surely the most confusing and immunochemically poorly based explanation of immune cross-reactivity between different member drugs with a common basic structure, the original A, B, C, D CS classification was recently "simplified" into three groups. The *D* ring is said to have an important role in CS cross-reactivity as a result of amino acids (such as arginine) in skin proteins binding at C21. After the initial subdivision of the CSs into the four groups, group D was further divided into two subgroups D1, the "stable" esters, and D2, the "labile" esters. It has been hypothesized that non-methylated CSs are implicated in sensitization to CSs after selective reaction with arginine to form stable cyclic adducts and, with this in mind, C16-methylated and non-methylated CSs were compared in patch tests on patients with proven CS contact allergy. Results showed that significantly more positive reactions occurred with groups A and D2 CSs without C16 methyl substituents than with groups C and D1 molecules

carrying a C16 methyl group. This led to a new classification of CSs into three groups (1) CSs that produce most of the allergic reactions—CSs that are non-methylated and usually nonhalogenated and essentially made up of groups A and D2 plus budesonide. (2) The halogenated molecules with a C16/C17 *cis*-ketal/diol structure, that is, acetonide group B. (3) CSs that produce allergy rarely—halogenated and C16-methylated CSs, that is, groups C and D1. It remains to be seen if this is the last word on CS cross-reaction patterns and classification of the different patterns.

Some comments seem pertinent to this body of work on structure–activity relationships and cross-reactivities of topically applied CSs. In the first place, the conclusions should not be automatically applied to delayed and immediate reactions following systemic administration of CSs. Second, the fact that the above interpretations and conclusions resulted from work unconnected to type I and type IV systemic reactions indicates why the data used to understand cross-reactivities at the cutaneous level are so difficult to explain and categorize and why the presented analyses of the data are so complex and frequently, and differently, interpreted. Results obtained from patch testing can, at best, be semiquantitative and meaningful, and accurate comparisons of recognition and reactivities of individual CSs are not possible on a molar basis. This is unlike the situation where specific IgE antibodies or T cells (less easily) can be utilized for side-by-side comparisons of recognition of structurally similar and dissimilar drugs.

12.4 Immediate Reactions to Corticosteroids

Interestingly, the anti-inflammatory and immunosuppressive actions of CSs seen with delayed cutaneous reactions to the drugs do not seem to affect immediate reactions in the skin as shown by the failure of locally injected methylprednisolone to prevent or attenuate cutaneous reactivity induced by prick tests of histamine and the histamine liberator codeine. In fact, when injected at the prick test site, the locally administered CS was associated with a significant increase in the flare induced by both agents. Although these results cannot automatically be extrapolated to chronic urticaria, it seems likely that CSs are unlikely to prevent or to improve this condition. However, in the case of systemic immediate reactions, the situation may be different as discussed below (Sect. 12.4.2).

12.4.1 Incidence, Sensitization, Clinical Presentation, and Risk Factors

With an incidence estimated to be about 0.1–0.3 %, immediate reactions to CSs are fortunately rare given the widespread usage of these drugs in many different diseases and because the risk of anaphylaxis to CSs may not be considered by some clinicians who may think that progressing symptoms are due to insufficient dosage. There are over 100 published reports of immediate hypersensitivity reactions to CSs, including many life-threatening reactions, after oral and parenteral administration. Most reactions have occurred in adults with surprisingly few reactions in children even though administration of CSs to children is common. Immediate reactions have followed after IV, intramuscular, subcutaneous, and intra-articular injection although the IV route is the most frequent elicitor of reactions. Symptoms include angioedema, generalized urticaria, pruritus, bronchospasm, hypotension, dyspnea, sneezing, nausea/vomiting, decreased consciousness, respiratory arrest, and anaphylactic shock including death. Risk factors include asthma, particularly aspirin-sensitive asthmatics, and renal transplant patients but it remains unclear whether atopy and heavy or prolonged exposure are risks—in fact, factors predisposing patients to risk of immediate reactions to CSs seem little investigated.

12.4.2 Immediate reactions: Important/Interesting Findings and Observations Reported so Far

Some systemic reactions to CSs are pseudoallergic, and there are many cases in the literature

where clear evidence for a true type I or type IV reaction is lacking. Almost certainly some of these reactions are anaphylactoid in nature, and it has been pointed out that such reactions may result from infusion of high doses of CSs such as methylprednisolone. Unlike true anaphylactic reactions, anaphylactoid reactions to drugs may be related to both the dose of drug and its rate of delivery. With methylprednisolone, for example, doses of 11 mg–2 g have caused systemic reactions, but most reactions occur after administration of more than 250 mg. In high dose therapy (more than 30 mg/kg), the drug should be infused over 30 min; more rapid infusion significantly increases the chance of an anaphylactoid response.

As already mentioned, there is no shortage of reports of immediate reactions to CSs but most of the results add little new information relevant to improving diagnosis or elucidating underlying mechanisms. Much of the reported information is similar, including the predominance of methylprednisolone and hydrocortisone in provoking reactions; the spectrum of symptoms observed; the limited diagnostic investigations employed; and the frequent absence of clear conclusions on cross-reactions, but occasional skin test findings are noteworthy. Over 40 years ago, intradermal tests for hydrocortisone, prednisolone acetate, and prednisone were positive in a patient who experienced hives after oral and intra-articular CS administration but only after an interval of 3 h or more. This "delayed" positive skin response has been occasionally observed by others, a good recent example being in a 2008 study when an intramuscular challenge with 10 mg of methylprednisolone hemisuccinate precipitated an immediate reaction and a positive intradermal test in a prednisolone-allergic patient who was initially skin test negative to the drug when the test was read at 20 and 60 min earlier the same day. Subsequent intradermal tests with hydrocortisone, betamethasone, triamcinolone, and paramethasone and intramuscular challenges with hydrocortisone, betamethasone, and paramethasone all produced negative results. Unlike the findings with locally administered CSs at sites previously prick tested with histamine and codeine (see above), it seems that immunosuppressive and/or anti-inflammatory actions of the methylprednisolone delayed the positive response at the skin test site initially negative at 60 min. The delayed appearance of positive skin tests to CSs suggests that it may be prudent to read such tests after an extended period, for example, 90 min or 2 h or more, if earlier readings at 20 and 60 min prove negative. In attempting to explain the appearance of the positive skin test to prednisolone after the intramuscular challenge with the drug, the investigators speculated that, unlike with the challenge test, the amount of drug injected intradermally was too small to form enough hapten–protein antigenic complex by binding to arginine on human albumin.

To overcome their poor solubility, CSs are often esterified at position C21 and used as the succinate, phosphate, or other esters. Succinate esters appear to be more immunogenic, but this has not been unequivocally established and no mechanism of increased antigenic/allergenic potential has been convincingly argued or demonstrated. As for CSs in general, it is thought that CS esters act as haptens in larger molecular weight complexes with carrier molecules. The question of cross-reactivity between CSs in immediate reactions has not always been clear. The rather complex cross-reactivity story elaborated for the topical CSs does not seem to apply for systemic reactions and different patterns of cross-reactivity, not easily categorized, have been observed. Cross-reactions between steroids with similar chemical structures are seen but often they belong to different groups used in the topical CS classification. Other reports describe cross-reactions between group A CSs, others failed to detect any cross-reactivity, and recognition of different succinate esters has also been noted. In the latter category, a patient who experienced an IgE-mediated anaphylactic reaction to methylprednisolone-21-succinate sodium proved prick test positive to this drug and to prednisolone-21-sodium succinate but not to prednisolone (i.e., the unesterified drug) or to betamethasone-21-dihydrogen phosphate, and oral challenges with both of these drugs were well tolerated.

The succinate ester grouping was suspected to be allergenically important since other nonesterified CSs and CSs with groups other than succinate at C21 were negative in both prick and challenge tests. Wide cross-reactivity between CSs could indicate immune recognition of the steroid backbone of the CS drugs whereas lack of cross-reactivity with other CSs is probably a consequence of recognition of individual, and sometimes unique, structural features.

12.4.3 Identifying and Understanding Cross-Reactions: Finding a Safe Alternative Corticosteroid

At the clinical level, negative skin and challenge tests can identify safe and alternative CSs for patients who have experienced a CS-mediated immediate allergic reaction. If cross-reactions, weak or strong, are identified in skin tests, systematic challenge testing might be employed, but the risks of challenge testing with even weakly cross-reacting drugs must be very carefully considered. If the risk to benefits ratio is judged to be in the patient's best interest, extreme care with all precautions should be followed in the provocation testing (see Sect. 4.4). In any case, for systemic reactions to CSs, further studies are needed on the sensitivity, specificity, and positive and negative predictive values of skin tests and, for a better understanding of clinical cross-reactions between CSs, these studies are a necessary prelude. For an understanding of cross-reactions in structural terms, quantitative immunochemical investigations are necessary to identify fine structural features recognized on individual CSs and to have any chance of anticipating and predicting possible reactions. With reactions mediated by drug-reactive IgE antibodies, hapten inhibition studies employing selected CSs, analogs, and other carefully chosen structurally relevant compounds in hapten inhibition experiments can provide a quantitative comparison of inhibitory potencies and thus a spectrum of cross-recognition from those CSs that are strongly cross-reactive to those that are completely unrecognized by the sensitizing antibodies.

Table 12.1 Skin test concentrations for some commonly used corticosteroid drugs

Corticosteroid	Prick test (mg/ml)	Intradermal test[a] (mg/ml)
Betamethasone phosphate	4	0.04
Budesonide[b]	0.25	0.0025
Dexamethasone phosphate	4	0.04
Hydrocortisone succinate	100	1
Methylprednisolone succinate	40	0.4
Prednisolone succinate	10[c]	0.1
Prednisone	30	–
Triamcinolone acetonide	40	0.4

Sensitizations to the excipients carboxymethylcellulose and macrogel have been observed in some patients with corticosteroid hypersensitivity

[a]Some investigators use up to a maximum of 10× these concentrations, i.e., a 1–10 dilution of the prick test concentrations

[b]Commonly used in patch tests at 0.01 % in petrolatum and 0.002 % in ethanol

[c]Up to 30 mg/ml has been used

12.4.4 Diagnostic Methods

Skin testing, both prick and intracutaneous, with appropriate concentrations of drugs together with challenge tests are the basis of the diagnosis of immediate systemic reactions to CSs. The recommended skin test concentrations are set out in Table 12.1, but the clinician should, as always, be aware that smaller concentrations may sometimes be advisable in the occasional patient showing exquisite sensitivity. For intradermal testing, suspensions of drug should not be injected. Specific IgE antibodies to CSs have rarely been described, probably partly because an appropriate assay has not generally been available, although a Phadia test for the detection of IgE antibodies to methylprednisolone-21-succinate sodium has been successfully applied. Note that a negative finding in a test for CS-reactive IgE does not necessarily rule out immediate hypersensitivity to a CS. Development of IgE assays for many CSs faces the technical problem of poor solubility of many of the CSs. Another potential difficulty may be the presentation of the CS in an appropriate haptenic form, which is the drug-carrier complex form involved in the initial sensitization and/

or immediate allergic reaction. The basophil activation test offers another potential test for the detection of an immediate reaction, and it has been successfully employed in at least three separate diagnostic investigations in different centers. In one case, CD63 expression was measured after the patient's blood was incubated for 30 min with methylprednisolone (0.1 μg/ml). The drug activated 20.2 % of the basophils compared to 6 % in control samples for a stimulation index of 3.4.

Summary

- Despite their anti-inflammatory and immunosuppressive properties, CSs can provoke both immunological and inflammatory responses, occasionally inducing immediate type I and delayed type IV allergic reactions. Type II cytotoxic and type III immune complex reactions do not appear to have been reported.
- For reactions after topical application, frequencies of from 0.2 to 5.98 % are claimed. For systemic reactions, the incidence of reactions is 0.1–0.3 %.
- The reaction of steroid glyoxals with arginine seems to be important for allergenicity of CSs since those CSs with the greatest capacity to bind to arginine also make the strongest allergens.
- Allergic contact eczema is the most common delayed hypersensitivity reaction to CSs. Other delayed reactions include maculopapular rash, exanthematous, papulo-vesicular and flexural rashes, rash with or without bullae or purpura, acute generalized exanthematous pustulosis, and erythema multiforme.
- Type IV reactions to CSs are mainly diagnosed by patch testing. Tixocortol pivalate, budesonide, and hydrocortisone 17-buyrate are the principal drugs employed for testing. A combination of tixocortol pivalate and budesonide detects over 91.3 % of CS-allergic patients.
- Because the anti-inflammatory effect of CSs can mask allergic reactions, too high a concentration of test agents may give false negative reactions, and this has led to the recommendation of extended reading times. The so-called 'edge effect' is a major factor to consider in reading patch test results for CSs.
- ELISPOT assays for cytokines such as IFN-γ appear to be potentially promising techniques to aid the diagnosis of delayed reactions to CSs.
- CSs have been classified into four structural groups A, B, C, and D on the basis of clinical cross-reactivity patterns obtained from patch test results. Drug examples corresponding to each group are: Group A, tixocortol pivalate; group B, triamcinolone acetonide; group C, betamethasone; group D, hydrocortisone-17-butyrate. After the initial subdivision of the CSs into the four groups, group D was further divided into two subgroups D1, the "stable" esters, and D2, the "labile" esters.
- A recent new classification divides the CSs into three groups (1) CSs that produce most of the allergic reactions—CSs that are non-methylated, usually nonhalogenated and essentially made up of groups A and D2 plus budesonide. (2) The halogenated molecules with a C16/C17 *cis*-ketal/diol structure, that is, acetonide group B. (3) CSs that produce allergy rarely—halogenated and C16-methylated CSs, that is, groups C and D1.
- Anti-inflammatory and immunosuppressive actions of CSs seen with delayed cutaneous reactions to the drugs do not seem to affect immediate reactions in the skin.
- Wide cross-reactivity between CSs probably indicates immune recognition of the steroid backbone of the CS drugs whereas lack of cross-reactivity with other CSs is probably a consequence of recognition of individual, and sometimes unique, structural features.
- Skin testing, both prick and intracutaneous, with appropriate concentrations of drugs together with challenge tests are the basis of the diagnosis of immediate systemic reactions to CSs.

Further Reading

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Drugs Used for Chemotherapy 13

Abstract

Many of the drugs used for chemotherapy have been, and still are, alkylating agents, antimetabolites, organoplatinum compounds, cytoskeletal disruptors, or anthracyclines, all agents with relatively broad rather than targeted and specific modes of action. The tyrosine kinase inhibitor imatinib mesylate and proteasome inhibitor bortezomib are recent examples of a more specific treatment strategy. Up to 30 % of patients develop acute infusion reactions to taxanes. Hypersensitive cross-sensitivity between docetaxel and paclitaxel is ~90 %. Most reactions to platinum drugs appear after multiple treatment cycles (usually at least six). Reactions are mainly type I or type IV hypersensitivity responses with a few cases of type II and type III hypersensitivities. The drug imatinib mesylate that inhibits both the ABL and BCR-ABL tyrosine kinases has been successful in treating chronic myeloid leukemia. In the chronic phase of treatment, neutropenia results in 35–45 % of cases, thrombocytopenia in 20 %, and anemia in 10 % of cases. Gefitinib and erlotinib are EGFR inhibitors, inhibiting the receptor's tyrosine kinase domain. Main hypersensitivities to both drugs include cutaneous reactions. GI symptoms, thrombocytopenia, peripheral neuropathy, and neuropathic pain are the most common side effects of the proteasome inhibitor bortezomib. Adverse cutaneous reactions to the drug are numerous.

Although a strictly literal meaning of "chemotherapy" would include, for example, treatment of an infection with an antibiotic or administration of aspirin for a headache, the word has become synonymous with drug treatment of cancers. Until fairly recently, chemotherapy with antineoplastic drugs has been largely based on the broad, nonspecific strategy of killing rapidly dividing cancer cells even though some other normal, healthy rapidly dividing cells (e.g., in the bone marrow, mucosal linings, and hair follicles) also undergo collateral harm. Recently, more selective strategies in which drugs are targeted to cancer cells, for example, by recognition of specific cellular antigens, have begun to be employed. In keeping with the universal dread of cancer, its widespread occurrence in so many different forms in humans of all ages, its high

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_13, © Springer Science+Business Media, LLC 2013

profile in medical research, and the vast amounts of money invested in attempts for a "cure," it is not surprising that numerous drugs have been employed for treatment of the many different cancers that occur in humans. Even a quick perusal of the medical literature reveals in excess of 100 drugs currently administered in significant quantities to cancer patients somewhere in the world. Of course, despite being less expensive, some of these drugs therapies are older, less specific in their action, and often less effective than some newer, more targeted therapies, and, for the immediate future, many long-established anticancer drugs seemed destined to be used less and less.

We are interested here in covering some of the most important currently used, and new, anticancer drugs and the adverse/hypersensitivity reactions they elicit.

In the identification and development of new anticancer drugs over many years, many of the compounds that have found application in the clinic have cytotoxic properties arising from a small number of different mechanisms of action. Many of the drugs used for treatment of malignant cells have been, and still are, alkylating agents, antimetabolites, organoplatinum compounds, cytoskeletal disruptors, or anthracyclines, all agents with relatively broad rather than targeted and specific modes of action. Table 13.1 summarizes some important properties of, and adverse reactions to, a selection of 17 anticancer drugs currently commonly used. Alkylating agents such as busulfan and cyclophosphamide and platinum-based agents like cisplatin that cross-link DNA; inhibitors of mitotic cell division by taxanes such as paclitaxel; drugs that intercalate with DNA like daunorubicin; pyrimidine analogs including 5-fluorouracil; and other antimetabolites such as pemetrexed represent a broad group of drugs that can be compared and contrasted with some recently introduced agents where a more refined strategy of specific action on a particular cancer cell rather than nonspecific inhibition and killing has been used. The tyrosine kinase inhibitor imatinib mesylate and proteosome inhibitor bortezomib are examples of the latter approach. Adverse reactions to these drugs will be discussed in detail together with reactions to some of the more important other groups of chemotherapeutic drugs currently widely used.

13.1 Taxanes

Taxanes are diterpenes and the name taxane is derived from the fact that these compounds were found to be produced by plants of the yew genus *Taxus*. The drug paclitaxel is so named because it was originally identified in the bark of the Pacific yew tree. Because of the difficulties involved in their synthesis (paclitaxel for example, has 11 chiral centers), natural products remain a source for some taxanes. Docetaxel is a semi-synthetic compound produced from a precursor found in the needles of the European yew tree. The taxanes are mitotic inhibitors, disrupting microtubule function so adverse reactions, including hypersensitivity responses, to these drugs when given as anticancer agents might be expected. In a women's cancer program retrospective study covering the period 1999–2004, severe hypersensitivity reactions occurred in 16 of 718 patients (2.2 %) given paclitaxel and in 9 of 93 patients (9.7 %) who received docetaxel. Up to 30 % of patients have been found to develop acute infusion reactions to taxanes. Acute hypersensitivity reactions are marked by urticaria, flushing, rashes, dyspnea, gastrointestinal symptoms, hypo-, and hypertension and back pain, the latter not yet well understood. Taxanes present difficulties in their formulation since they are poorly soluble in aqueous media and their consequent presentation in vehicles such as Cremophor also contribute to adverse reactions. Multiple mechanisms may underlie infusion reactions to taxanes. Reactions may be IgE antibody-mediated or due to direct mast cell/basophil or complement activation. The similarity in structure between docetaxel and paclitaxel suggests cross-reactions and cross-sensitivity. Some early results on small numbers of patients indicated successful substitution of docetaxel for paclitaxel, but this conclusion was not supported in the later women's cancer study mentioned above where ten patients with severe hypersensitivity to paclitaxel also reacted to docetaxel, giving a cross-sensitivity rate of 90 %. The different vehicles used for the two drugs indicated that the cross-reactions were probably due to the taxanes and not the vehicles.

Table 13.1 Important non-mAb anticancer (chemotherapeutic) agents and their adverse/hypersensitivity reactions

Type of chemotherapy drug and generic name	Chemical class	Cancer indications	Mechanism of action	Adverse/hypersensitivity reactions		Incidence/risk factors/ diagnosis/comments
				Cutaneous	Systemic	
Alkylating agents						
Busulfan	Alkylsulfonate	Chronic myelogenous leukemia	Alkylation producing guanine–adenine cross-linking	MER; U; hyperpigmentation; EM	Pulm. fibrosis; Tc; leukopenia; anemia; seizures	Periodic blood counts for bone marrow suppression
Chlorambucil	*N* mustards. Chloroalkylamine	Chronic lymphocytic leukemia	Nonspecific DNA alkylating agents	EM; TEN; CD; Ex; U; St	Fever; interstitial pn; immune hemolytic anemia; bms;Tc	Fairly well tolerated. Type III immune complex reactions? Diagnosis[a]
Cyclophosphamide	*N* mustard.[b] Oxazophorine	Lymphomas, brain, leukemia	Forms DNA cross-links	U; Angio; rash; EM; SJS; TEN; Vasc	Anaph; Br; bms	Some IgE-mediated reactions[c]? Diagnosis: SPT and IDT 1 mg/ml
Mitotic inhibitors						
Docetaxel[d,e]	Taxane (Diterpene)	Breast, prostate, ovarian, nonsmall cell lung	Inhibition mitotic cell-division	Acral erythema; scleroderma-like; St; alopecia	Neutropenia; anemia; Dys; Br; GI symptoms; back pain	Cross-reaction with paclitaxel but reactions less frequent. Infusion reactions—C′ activation, IgE, mast cell/basophil action[f]?
Paclitaxel[d,e,g]	Taxane (Diterpene)	Breast, ovarian, head and neck, lung, bladder, etc.	Inhibition mitotic cell division	Acral erythema; injection site reaction; AGEP-like; EM; SJS	Neutropenia; hypersensitivity pn; GI symptoms; back pain; Dys	Incidence 10–16 % without premedication. Risks—atopy, bee sting allergy
Platinum-based agents[h]						
Carboplatin	Organoplatinum	Ovarian, lung, head and neck, breast, testicular, brain (children)	Cross-links DNA	Rash; Pr; U; Angio; erythema; flushing	Anaph; Dys; Br; CV and GI symptoms	Incidence 12 % (1 % < 6, 27 % > 7 courses). Deaths reported. Diag. SPT and IDT. Some reactions IgE-mediated?
Cisplatin	Metal coordination complex	Sarcomas, some carcinomas (small cell lung, ovarian), lymphomas, germ cell	Cross-links DNA	Rash; flushing; Pr; U	Anaph; Dys; Br	Incidence 5–20 %. Repeated exposure before onset of symptoms. Diagnosis: SPT and IDT[i]
Oxaliplatin	Organoplatinum	Colorectal (admin. with fluorouracil and leucovorin)	Cross-links DNA	Pr; U; Angio; rash; erythema; flushing; acral erythema	Anaph; fever; Dys; GI symptoms; types II and III hypersensitivities	Incidence 10–19 %. Reactions after 7–9 courses. Diag. SPT and IDT. Some IgE-med. reactions?

(continued)

Table 13.1 (continued)

Type of chemotherapy drug and generic name	Chemical class	Cancer indications	Mechanism of action	Adverse/hypersensitivity reactions		Incidence/risk factors/ diagnosis/comments
				Cutaneous	Systemic	
Anthracycline antibiotics						
Daunorubicin	Anthracycline	Acute myeloid and lymphocytic leukemias, neuroblastoma	Intercalates with DNA	Rash; U; Angio	Anaph; fever; cardiac toxicity; bms	Reactions uncommon (1–2 %). Cross-reaction with doxorubicin?
Doxorubicin[j]	Anthracycline	Hematological, ovarian, Kaposi's	Intercalates with DNA	Pr; U; Angio; acral erythema; rash; St; injection site reactions	Anaph; hypersens. infusion reaction; Br; cardiotoxicity	Reactions uncommon (U, 0.6–3 %). Hypersensitivity reaction 8 %
Tyrosine kinase inhibitors[k]						
Imatinib mesylate[l]	Phenylaminopyrimidine derivative	Chronic myeloid leukemia, GI stromal tumors, and other cancers	Inhibits BCR-ABL tyrosine kinase	Rash; Angio; Pr; SJS; AGEP; DRESS; Vasc	Hypersensitivity pn; neutropenia; tumor lysis syndr; anemia; hepatotoxicity	Incidence 31–44 % cutaneous reactions. 21 % mild-moderate reactions. Severe exfoliative rash 1–500
Proteosome inhibitor						
Bortezomib[m]	Boronic acid derivative of *N*-protected dipeptide	Multiple myeloma, non-Hodgkin, mantle cell lymphoma	B atom binds to 26S proteasome	Erythematous plaques; rashes; Vasc; TEN	Peripheral neuropathy; Neutropenia; Tc; Dys; cardiac disorders; Tc	Rash 8–18 %
Antimetabolites: *Pyrimidine analogs*[n]:						
Capecitabine	Derivative of 5-fluorouracil	Colorectal, pancreatic, metastatic breast, stomach	Prodrug form of 5-fluorouracil	Acral erythema; erythema; palmar-plantar keratoderma; exfol. dermatitis; St	CV; GI; hematologic; cytopenias	Not to be used with leucovorin
5-Fluorouracil	Pyrimidine	Colorectal, pancreatic, breast	Thymidylate synthase inhibitor	Acral erythema; maculopap. rash; inj. site reaction; CD; St	Anaph; diarrhea; myelosuppression; GI; mucositis	Palmar-plantar dermatitis risk factor. Diag.—IDT and patch (with care) IgE-mediated? Direct cytotoxic effect
Gemcitabine[o]	Nucleoside analog	Pancreatic, lung, ovarian, breast, bladder	Replaces cytidine during DNA replication	Maculopap. rash; acral erythema; Pr; bullous dermatitis; SJS; TEN; Vasc; St	Fever; flu-like symptoms; hypersensitivity pn; diarrhea; cytopenias	–
Antimetabolites: *Anti-folates*:						
Methotrexate[p]	An amino-pteroylglutamic acid	Breast, head, neck, lung, bladder, leukemia, lymphoma	Inhibits dihydro folate reductase	Rash; acneiform papular and nodular eruptions; acral erythema; EM; SJS; TEN; Vasc; St; U	Anaph; low white cell count; Br; hem anemia; hepatotoxicity; GI	Types I[q], II (hemolytic anemia), and III (cut. Vasc.) Diagnosis: SPT and IDT[q]

Pemetrexed	Pyrrolopyrimidine	Lung, mesothelioma	Inhibits folate-dependent enzymes	Rash; Pr; TEN; U; Vasc; St; mucositis	Low blood count; diarrhea; nausea and vomiting; Dys	Incidence of cutaneous reactions 14%[r]

AGEP acute generalized exanthematous pustulosis; *Anaph* anaphylaxis, *Angio* angioedema, *bms* bone marrow suppression, *Br* bronchospasm, *CD* contact dermatitis, *CV* cardiovascular, *DRESS* drug reaction with eosinophilia and systemic symptoms; *Dys*, dyspnea; *EM* erythema multiform, *Ex* exanthema, *GI* gastrointestinal, *MER* macular erythematous rash, *pn* pneumonitis, *Pr* pruritus, *SJS* Stevens–Johnson syndrome, *St* stomatitis, *Tc* thrombocytopenia, *TEN* toxic epidermal necrolysis, *U* urticaria, *Vasc* vasculitis

[a]Lymphocyte stimulation test positive in a few cases. Challenge test often positive but may be harmful

[b]Potential cross-reactivity with other *N* mustards due to common bischloroethylamine group

[c]Can form antigenic complexes with protein

[d]Desensitization generally well tolerated and successful within 6–7 h (see text)

[e]Premedication with oral corticosteroids often used (see Sect. 13.1.1)

[f]Vehicle polysorbate 80 implicated in some reactions

[g]Cremophor vehicle associated with some reactions involving C′ activation

[h]Premedication and desensitization employed (see Sect. 13.2.4)

[i]See Sect. 13.2.3

[j]Premedication with corticosteroids and antihistamines. Slow infusion rate (0.1–0.2 mg/min) required

[k]See also gefitinib and erlotinib in Sect. 13.3.3

[l]For medication with prednisolone to achieve tolerance of therapeutic dosage and desensitization see Sect. 13.3.2

[m]Premedication with corticosteroids

[n]Some patients unable to metabolize pyrimidine drugs (~8 % of population) may have dihydropyrimidine dehydrogenase (DPD) deficiency

[o]Structurally related to cytarabine

[p]Often symptoms despite premedication. Desensitization sometimes employed

[q]Over 20 reported cases of anaphylaxis. Skin prick test conc. 10 mg/ml

[r]To prevent skin rashes, dexamethasone 4 mg b.i.d. the day before, the day of, and the day after administration

13.1.1 Premedication for Taxanes

There is a significant risk of hypersensitivity reactions following taxane administration and to minimize this, patients require premedication. Both docetaxel and paclitaxel are usually administered once every 3 weeks with docetaxel being infused over 1 h and paclitaxel over periods of from 1 to 96 h. The premedication regimen for patients receiving docetaxel consists of oral dexamethasone 8 mg twice daily for 3 days starting 24 h prior to the commencement of infusion. For paclitaxel infused over 1, 3, and 24 h, antihistamines are administered as well as the steroid. The H_1 antagonist diphenhydramine (50 mg) and an H_2 antagonist (cimetidine 300 mg, ranitidine 50 mg, or famotidine 20 mg) are given IV prior to infusion beginning while oral dexamethasone 20 mg is administered 12 and 6 h prior. There is some evidence that premedication is not required for infusions extending over 96 or more hours. The corticosteroid is used to prevent hypersensitivity reactions, decrease fluid retention, and decrease skin and nail adverse effects. Note that the dose-limiting toxicity of docetaxel and paclitaxel is febrile neutropenia; docetaxel 100 mg m^{-2} administered over 1 h every 3 weeks is associated with neutropenia in ~75 % of patients; for paclitaxel 135–300 mg m^{-2} over 24 h the figure is 52 %. Another published premedication protocol that claimed to limit the development of acute hypersensitivity to docetaxel consists of oral methylprednisolone 32 mg, cetirizine 10 mg, and ketotifen 1 mg, 12 and 3 h before infusion. For taxanes given weekly, the efficacy and safety of premedication were assessed in the treatment of nonsmall cell lung cancer. Weekly administrations of docetaxel and paclitaxel were assessed as safe and active protocols. For docetaxel, oral dexamethasone 4.5–7.5 mg twice daily for the day before, the day of, and the day after, together with IM promethazine 25 mg and IV cimetidine 600 mg 30 min before docetaxel are recommended. For paclitaxel the recommended protocol is: dexamethasone 2.25–7.5 mg orally 12 and 2 h before, and promethazine 25 mg IM and cimetidine 600 mg IV 30 min before the taxane. For weekly taxane dosage, the question of whether or not doses of dexamethasone can be reduced is important for patients experiencing, or at high risk of, steroid-induced side effects. Some have claimed that the optimal schedules remain to be determined and larger prospective clinical trials are needed.

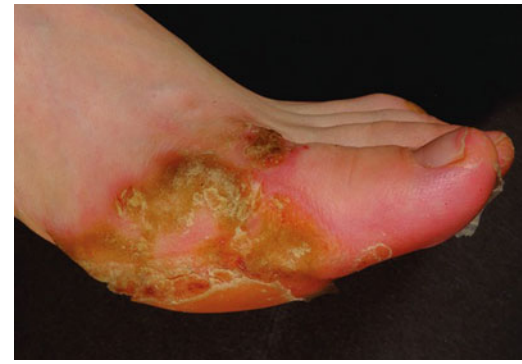

Fig. 13.1 Hand-foot skin reaction in a patient given sorafenib, a targeted inhibitor of some tyrosine kinases (including VEGFR and PDGFR) and Raf kinases approved for treatment of renal cell and hepatocellular carcinomas. Note that hand-foot skin reaction is a distinct entity from hand-foot syndrome or acral erythema which manifests as erythema, swelling, and desquamation of the palms and soles in cancer patients mainly during non-targeted chemotherapy. Drugs most commonly implicated in the latter reaction include 5-fluorouracil, capecitabine, cytarabine, pegylated doxorubicin, and also sorafenib and sunitinib, but a range of other chemotherapeutic agents including docetaxel, paclitaxel, oxaliplatin, gemcitabine, and methotrexate are also known causes. Both sorafenib and sunitinib provoke a high incidence of hand-foot skin reaction in patients (Reproduced with permission from Hauschild A, Kähler KC, Egberts F in Leong, SPL, editor. From Local Invasion to Metastatic Cancer, 1st ed. New York: Springer, 2009)

A recent multi-institution survey in Japan drew attention to an important effect of histamine H_2 antagonists on docetaxel-induced skin toxicity. Analyses revealed that administration of H_2 blockers was associated with a significantly higher incidence of acral erythema (hand-foot syndrome; palmar-plantar erythrodysesthesia; compare with hand-foot skin reaction, Sects. 13.3.2 and 13.3.3 and see Fig. 13.1) and facial erythema. Steroids and H_2 blockers affect the metabolism of docetaxel by cytochrome P_{450}3A4 (CYP3A4), but dexamethasone dosage did not change the incidence of hand-foot syndrome or facial edema.

13.1.2 Desensitization for Hypersensitivity Reactions to Taxanes

In the rapid desensitization protocol for paclitaxel published by Sullivan (Sullivan TJ. Protocols for rapid and slow drug allergy desensitization, Atlanta, Georgia, 2009), three concentrations of drug are employed—full strength solution, that is 300 mg paclitaxel in 500 ml in physiological saline (0.6 mg/ml); a 1 in 10 dilution (0.06 mg/ml); and a 1 in 100 dilution (0.006 mg/ml). Except for the last dosage step, each of the 11 preceding steps is infused for 15 min before changing to the next dose. Beginning with the 1 in 100 dilution (0.006 mg paclitaxel/ml) and an infusion rate of 2 ml/h, the solution is infused for 15 min followed by infusion rates of 5, 10, and 20 ml/h, respectively for steps two, three, and four. For the next four steps (numbers five to eight, again at 15 min intervals), the one in ten dilution of drug (0.06 mg/ml paclitaxel) is infused at rates of 5, 10, 20, and 40 ml/h, respectively. At steps 9, 10, and 11, full strength solution (0.6 mg paclitaxel/ml) is infused at 10, 20, and 40 ml/h, respectively. For the last step (step 12), full strength solution is infused at 80 ml/h until the remaining full strength solution has been given.

The Castells group treated 17 consecutive patients with hypersensitivity to taxanes using a standard 6–7 h desensitization protocol. The patients underwent a total of 77 rapid desensitizations to docetaxel or paclitexal. Seventy two of the procedures were tolerated without reactions, four patients responded with hypersensitivity reactions that were milder than their initial reactions, and these patients tolerated re-administration of infusions. Five patients rechallenged before desensitization experienced recurrent reactions even though they were given additional premedication, and the infusion rate was reduced.

13.2 Organoplatinum Chemotherapeutic Drugs

Platinum-based cytotoxic compounds that cross-link DNA are some of the most active and effective cytotoxic drugs for the treatment of ovarian and almost all of the common tumors except breast and prostate tumors. Treatments with platinum drugs is often effected in combination with other anticancer agents and, overall, it is estimated that up to 50–70 % of cancer patients are treated with platinum drugs. Cisplatin, the first of the so-called organoplatinum drugs, contains no organic component and is, in fact, a metal coordination compound with a square-planar platinum (II) center coordinated to two ammonia and two chlorine ligands in a *cis*-ligand conformation (Fig. 13.2). The success of cisplatin in the clinic focused research interest on other possible platinum chemotherapeutic drugs and led to the general rule that a neutral square-planar platinum (II) center containing two *cis*-amines and two leaving groups are required for good anticancer activity. The first follow-up drug to gain worldwide clinical acceptance was carboplatin that contains the *cis*-$Pt(NH_3)_2$ active group of cisplatin with the chloride leaving groups replaced by a bidentate dicarboxylate (Fig. 13.2). Carboplatin has a similar anticancer profile as cisplatin but, although it is just as effective for ovarian cancer, its potencies against head, neck, and testicular cancers are less. On the other hand, carboplatin evokes fewer side effects, and this has generally made it the drug of choice over cisplatin. In 2004 oxaliplatin became the third widely accepted and used platinum drug. Oxaliplatin, unlike the other two platinum chemotherapeutics, is effective against colorectal cancer and, in addition, it is active against some cisplatin-resistant cancers. In the structure of oxaliplatin, the amines are part of the 1,2-diaminocyclohexane framework (Fig. 13.2).

13.2.1 Symptoms of Hypersensitivity to Platinum Drugs

Exposure to platinum salts, especially in miners and other industrial workers, has been known to provoke hypersensitivity reactions since, at least, the 1940s while reactions to platinum therapeutic agents, viz. cisplatin, were first described in the 1970s. Symptoms to the drugs may develop during infusion within minutes or after hours or

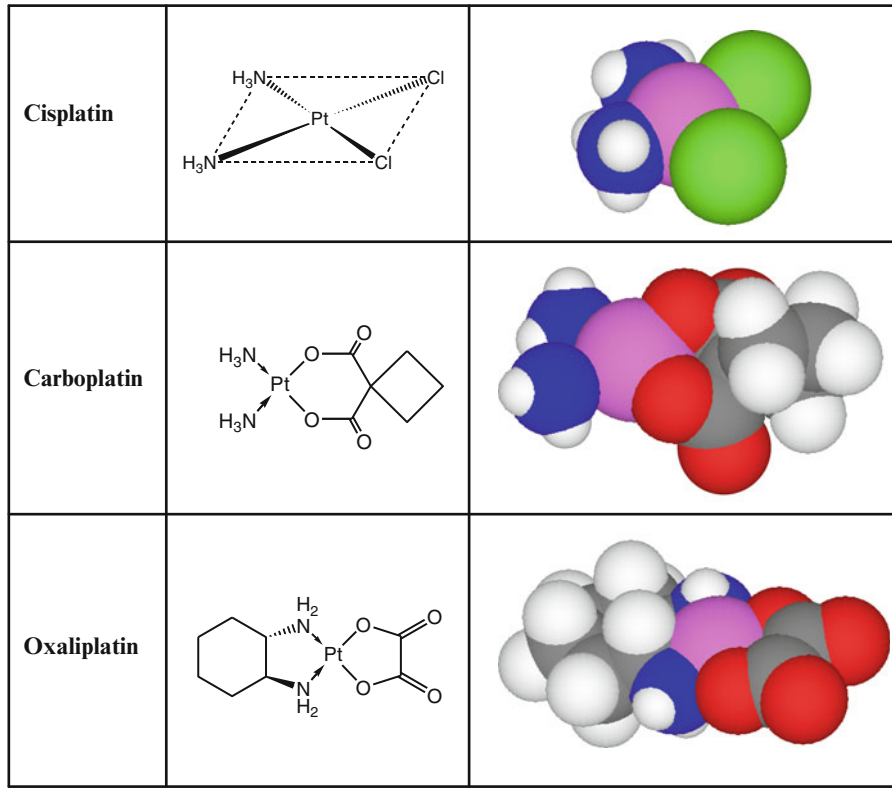

Fig. 13.2 Two- and three-dimensional structures of platinum-based chemotherapeutic drugs. Cisplatin, the first of the so-called organoplatinum drugs, contains no organic component. It is a metal coordination compound with a square-planar platinum (II) center coordinated to two ammonia and two chlorine ligands in a *cis*-ligand conformation. Carboplatin contains the *cis*-$Pt(NH_3)_2$ active group of cisplatin with the chloride leaving groups replaced by a bidentate dicarboxylate. Oxaliplatin also has the square planar platinum center but, unlike cisplatin and carboplatin, it has the bidentate ligand 1,2-diaminocyclohexane instead of two monodentate ammine ligands as well as a bidentate oxalate group

days with a mild rash being the first manifestation. The clinical features of the reactions are highly variable—in one survey of carboplatin-induced hypersensitivity, 100 % of patients had cutaneous symptoms (mainly palmar or facial flushing), 57 % had cardiovascular symptoms, 42 % gastrointestinal disturbances, and 40 % respiratory symptoms. Commonly seen mild reactions are rash, urticaria, flushing, palmar itching, a burning feeling, hand and facial edema, pruritus, back pain, abdominal cramping, and diarrhea. These symptoms usually resolve quickly with antihistamines and steroids. Moderate to severe symptoms include diffuse erythroderma, tachycardia, chest tightness, wheezing, facial swelling, dyspnea, hypertension, or hypotension (Table 13.1). Other more severe symptoms sometimes reported are bronchospasm, chest pain, seizures, and systemic anaphylaxis that may be life-threatening. Reactions to oxaliplatin are similar to those seen in response to cisplatin and carboplatin, but the responses to oxaliplatin tend to be more heterogeneous and unpredictable with fewer cutaneous reactions; idiosyncratic reactions like cytokine release syndrome and pulmonary fibrosis; fewer reports of severe anaphylaxis; and a higher incidence of respiratory symptoms including laryngeal spasms and hypoxemia. A few cases of type II thrombocytopenia and type III immune complex-mediated urticaria, joint pain, and proteinuria associated with oxaliplatin have also been reported.

13.2.2 Incidences of, and Risk Factors for, Hypersensitivity Reactions to Platinum Drugs

For cisplatin, the overall incidence of reactions is 5–20 %, reactions occur within minutes of the commencement of infusion, most reactions occur between the fourth and eighth course, and reactions increase with concomitant radiation. The overall incidence of reactions to carboplatin has been reported as 1–44 % and, in another study, 9–27 %. Reactions occur within minutes or days of infusion; less than 1 % of hypersensitivity reactions result during cycles 1–5; 6.5 % occur during cycle six; 27 % are seen in cycle seven or more; and 44 % occur in third-line treatment. Approximately half of all reactions to carboplatin are moderate to severe. Reactions to oxaliplatin occur with an incidence of 10–19 % and manifest within minutes or hours of infusion. Again, most reactions appear after a number of treatment cycles (usually at least six).

Risk factors for the platinum chemotherapeutic drugs have not yet been thoroughly studied and well defined. Most information so far has been obtained for carboplatin. Apart from the already clearly established risks of the number of prior treatments with platinum drugs and a high rate of drug infusion, other suggested risk factors so far include a history of drug allergy, a carboplatin-free interval of greater than 13 months, patients with ovarian cancer, children receiving weekly carboplatin infusion rather than monthly infusions, and the female gender. The antineoplastic drug used in combination with the platinum drug can also influence the incidence of hypersensitivity reactions to the platinum agent. For example, the CALYPSO study of the Gynecologic Cancer Intergroup showed that carboplatin with pegylated liposomal doxorubicin produced fewer reactions than the combination of the platinum drug with paclitaxel. Risk factors identified so far for oxaliplatin reactions include a young age, female gender, and use of the drug as salvage therapy.

13.2.3 Mechanisms and Diagnosis of Platinum Drug-Induced Hypersensitivity Reactions

Reactions are thought to be mainly type I or type IV hypersensitivity responses but, as mentioned above, a few cases of type II and type III hypersensitivities have been reported. Nevertheless, the complexity and unpredictable nature of many responses suggests that a number of mechanisms, both immune and nonimmune, may be operative in many reactions. A number of authors are sure that many reactions to the platinum drugs are consistent with type I, IgE antibody-mediated hypersensitivity. Although there has been no clear and unequivocal demonstration of the existence of IgE antibodies specifically directed at cisplatin, carboplatin, or oxaliplatin (including specific inhibition of antibody binding by the drug and structural analogs), previous findings of IgE antibodies to platinum salts in platinum-exposed workers, the appearance of sensitization only after multiple infusions, positive skin tests to carboplatin, and anaphylactic reactions are all taken as evidence of a type I hypersensitivity mechanism. Skin testing with the three platinum drugs has been employed to identify at-risk patients and predict platinum hypersensitivity but as yet it has not found widespread acceptance and application as a routine diagnostic procedure. In a study of patients with recurrent ovarian or peritoneal carcinoma who had received more than seven courses of carboplatin, intradermal testing with 100–240 μg of carboplatin revealed 13 of 47 patients (28 %) with a positive test. A negative skin test correctly predicted the absence of a hypersensitivity reaction in 166 of 168 courses of chemotherapy (98.8 %). Two patients experienced a reaction after showing a negative skin test. A follow-up study on 126 patients confirmed the association between a negative carboplatin skin test and no resultant severe hypersensitivity reaction after the next infusion, but the implications of a positive test remained less certain. Patch, prick, and intradermal tests with cisplatin, carboplatin, and oxaliplatin on 21 patients produced negative

patch test results in all 21 patients, five positive reactions in the prick test, and 12 positives in the intradermal test. Cross-reactions were observed in four cases, and delayed reactions occurred in three patients. It was concluded that the intradermal test was superior to the other two tests and its good negative predictive value allows safe re-treatment by detecting potential cross-reactions. Results from some other studies also support the superiority of the intradermal test. In response to the report of the three delayed reactions, it was pointed out that intradermal tests with platinum drugs have a good negative predictive value in immediate reactions, but the test can induce false positives with these drugs. Most skin test investigations have been on patients with carboplatin, but results and conclusions have not always been in agreement. Fifty three of sixty patients referred for carboplatin hypersensitivity were skin test positive to the drug, one patient showed a delayed positive reaction, two became positive after further infusions, and the remaining four skin test-negative patients experienced hypersensitivity during a subsequent infusion. Skin tests on 54 patients receiving re-treatment with carboplatin predicted hypersensitivity reactions in only 64 % of affected patients leaving the authors to conclude that skin testing did not reliably predict carboplatin-induced reactions. Skin test concentrations generally employed for carboplatin are 10 mg/ml in the prick test and 0.02 ml of 0.1 mg/ml in the intradermal test increasing in tenfold concentration steps to a maximum of 10 mg/ml. In some studies, a maximum skin test concentration of 3 mg/ml has been used for carboplatin. For oxaliplatin, a concentration of 1 mg/ml is used in the prick test and a maximum of 0.1 mg/ml in the intradermal test.

In summary, it has been claimed that skin tests are positive in more than 80 % of the platinum drug-treated patients tested and, when the test is negative, the risk of a hypersensitivity reaction is reduced sevenfold or even eliminated. This, in turn, has led to the recommendation that skin testing should be performed on every patient before the eighth drug infusion. Skin testing for oxaliplatin sensitivity has been claimed to be positive in 75–100 % of oxaliplatin hypersensitive patients. Skin testing may also help in ruling out cross-reacting drugs when substituting one platinum drug for another. Nevertheless, it has been argued that it is not practical to employ skin testing as a routine test in everyday clinical practice since prior experience in its execution is required, and hypersensitivity reactions might still occur even in the case of a negative skin test. The first of these objections cannot be sustained—if the simple skills of skin testing are lacking, the situation can, and should, be readily rectified by professionals who are charged with the responsibility of seeking and maintaining the best available diagnostic procedures. The possibility of reactions in skin test-negative patients is indeed likely but that, again, should always be seen as a possibility to be anticipated and managed and such a possibility is not a sufficient reason to forego the possible advantages that skin testing can provide.

13.2.4 Desensitization

Attempts to readminister the same drug or change to a different platinum drug can be dangerous, and desensitization to the drug is sometimes considered to be the best option. Desensitization protocols to the platinum drugs are not standardized, and a number of different protocols are currently employed in different institutions. Table 13.2 sets out a 12 step, ~ 6 h desensitization protocol for carboplatin in skin test-positive patients hypersensitive to the drug and receiving desensitization for the first time. Conversion of a positive skin test to carboplatin to a negative response after desensitization supports the existence of a specific IgE antibody-mediated response in patients. As well as carboplatin, a number of different desensitization protocols for oxaliplatin have been published, but the number of patients treated so far is small. The essential procedures of premedication, escalating dosage, infusion times, and duration of the procedure are similar to those employed for carboplatin. Drugs used for premedication are generally diphenhydramine or hydroxyzine as histamine H_1 inhibitors; famotidine, cimetidine, or ranitidine as H_2

Table 13.2 Desensitization protocol[a] for carboplatin hypersensitivity in patients undergoing desensitization for the first time

Step	Infusion rate (ml/h)[b]	Time infused (min)	Administered dose (mg)	Cumulative dose infused (mg)
1	2	15	0.010	0.010
2	5	15	0.025	0.035
3	10	15	0.050	0.085
4	20	15	0.100	0.185
5	5	15	0.250	0.435
6	10	15	0.500	0.935
7	20	15	1.000	1.935
8	40	15	2.000	3.935
9	10	15	5.000	8.935
10	20	15	10.000	18.935
11	40	15	20.000	38.935
12[c]	75[c]	184.4	461.065	500.00
Totals:		5 h 49.4 min	500 mg	

Data from Lee C-W et al. Gynecol Oncol 2004;95:370

[a]Conducted in intensive care unit, Brigham and Women's Hospital. β-Blockers withheld 1 day before. Informed consent obtained. All rescue medications on hand. Patients premedicated with diphenhydramine 25 mg, famotidine 20 mg IV 30 min before initiation of infusion

[b]Using appropriate concentrations to deliver the required dose in the required time

[c]A constant rate of infusion maintained to deliver the remainder of the total carboplatin dose

antagonists; and corticosteroids such as dexamethasone, prednisolone, and hydrocortisone. Oxaliplatin dilutions employed cover the range from 1:100,000 to 1:1 in from 5 to 13 steps over a total time range of from 5.8 to 8 h. Some protocols employ continuous fixed rate infusion extending over 24–48 h. In some procedures, magnesium sulfate and calcium carbonate have been added and claimed to increase the success rate of desensitization.

13.3 Tyrosine Kinase Inhibitors

Until relatively recently, chemotherapy has not discriminated effectively between rapidly dividing tumor cells and normal cells, in particular cells in the bone marrow and gastrointestinal tract. This has led to toxic effects for patients including an array of severe nonimmune adverse reactions as well as hypersensitivities ranging from mild skin manifestations to life-threatening cutaneous and systemic reactions. Effective targeted therapies with high specificity toward tumor cells but broad therapeutic application and absence of toxicity have always been desired, but their arrival (in the form of tyrosine kinase inhibition strategies), has only recently seemed likely. Tyrosine kinases have become recognized as important targets for cancer therapy because they have an important role in the modulation of growth factor signaling, and some tyrosine kinase inhibitors have been found to have antitumor activity and to be effective in treating some cancers in the clinic. This targeted strategy for killing tumor cells and some of the important drugs developed to achieve it will be discussed.

13.3.1 The Philadelphia Chromosome and Tyrosine Kinases

The Philadelphia translocation t(9;22)(q34;q11) or Philadelphia chromosome is a chromosomal defect resulting in gene fusion of the BCR and ABL genes. The BCR (**b**reakpoint **c**luster **r**egion) gene is on chromosome 22 (region q11) and the ABL (so named because the **A**bel**l**son leukemia virus has a similar protein) tyrosine kinase gene is on chromosome 9 (region q34). The resultant

fusion gene is the BCR-ABL oncogene. The Philadelphia chromosome is a cytogenetic abnormality seen in 95 % of chronic myeloid leukemia patients and 15–30 % of adults with acute lymphoblastic leukemia.

Tyrosine kinases can be classified as receptor and non-receptor kinases. They are enzymes that catalyze the transfer of the γ-phosphate group from adenosine triphosphate to the hydroxyl group of tyrosine on signal transduction molecules. This is an important activating step that leads to increases in tumor cell proliferation and growth. The oncogene BCR-ABL results in the expression of two forms of tyrosine kinases and a large increase in myeloid cell numbers. In summary, the BCR-ABL mutation is present in the great majority of chronic myeloid leukemia patients, the Bcr-abl fusion protein is unique to leukemic cells, it is expressed in high levels, and its tyrosine kinase activity is essential in the induction of leukemia. However, although tyrosine kinases were implicated as oncogenes in some animal tumors induced by retroviruses more than 30 years ago, it was not until the introduction of the drug imatinib that inhibits both the ABL and BCR-ABL tyrosine kinases and which has been successful in treating chronic myeloid leukemia that tyrosine kinases were regarded as good targets for cancer chemotherapy. In addition to imatinib, some inhibitors of receptor tyrosine kinases targeting epidermal growth factor receptor (EGFR; ErbB1; HER1; a member of the ErbB family of receptors), vascular endothelial growth factor receptors (VEGFR), and platelet-derived growth factor receptors (PDGFR) have been found to have antitumor and/or other activities. These receptor tyrosine kinase inhibitors include gefitinib, erlotinib (both inhibitors of EGFR), lapatinib (inhibits ErbB1 and ErbB2), vatalanib (inhibits VEGFR-1 and VEGFR-2), semaxinib (inhibits VEGFR-2), and leflunomide (inhibits PDGFR-mediated cell signaling, i.e., phosphorylation). Elevated EGFR tyrosine kinase activity is found in most solid tumors; following is a list of the percentage expression of EGFR by some of the most common human cancers: Nonsmall cell lung cancer 40–80, head and neck 80–100, gastric 33–81, colorectal 25–100, pancreatic 30–50, ovarian 35–70, breast 15–37, prostate 40–90, and glioma 40–92 %

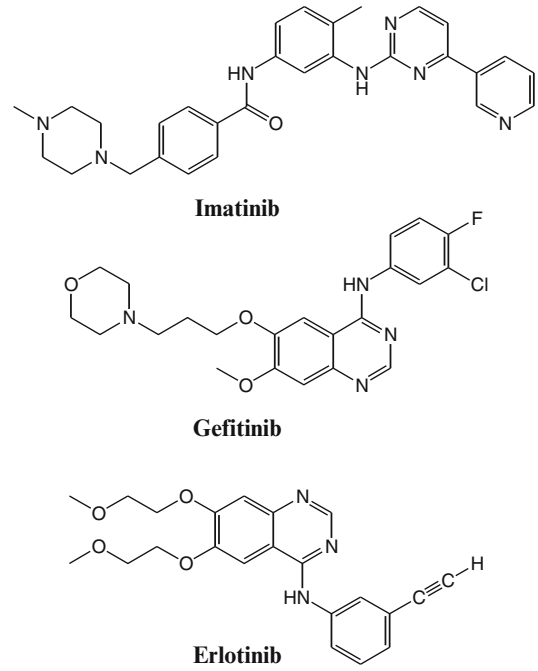

Fig. 13.3 Structures of the tyrosine kinase inhibitors: imatinib, a phenylaminopyrimidine derivative, and the 4-aminoquinazoline derivatives, gefitinib and erlotinib. Imatinib is marketed as the mesylate salt

13.3.2 Imatinib Mesylate

Used for the treatment of chronic myeloid leukemia, unresectable or metastatic gastrointestinal stromal tumors, and some other cancers, imatinib mesylate, a phenylaminopyrimidine derivative (Fig. 13.3), and some other targeted tyrosine kinase inhibitors are generally well tolerated with less severe systemic effects, especially when compared to most cytotoxic chemotherapies. Dermatologic reactions such as papulopustular rash, hand-foot skin reaction (Fig. 13.1), xerosis, pruritus, and mouth, hair, scalp, and nail abnormalities are the main adverse responses to many of the targeted drugs including gefitinib, lapatinib, erlotinib, regorafenib, pazopanib, sorafenib, and sunitinib.

Imatinib is marketed as the mesylate, that is, the salt of methanesulfonic acid (CH_3SO_3H). The most common non-hematologic adverse reactions to the drug include superficial edema, especially periorbital edema, nausea, vomiting, diarrhea, muscle cramps, myalgia, arthralgia, fatigue, abdominal pain, headache, and, most common of all, cutaneous reactions. Patients receiving standard dose imatinib therapy in the chronic phase of chronic myeloid leukemia experience neutropenia in 35–45 % of cases, thrombocytopenia in 20 % of cases, and anemia in 10 % of cases. Although most cutaneous reactions are mild and dose dependent, severe reactions such as Stevens–Johnson syndrome (SJS), exfoliative dermatitis, toxic epidermal necrolysis (TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS) have been reported. Other severe reactions to the drug include rare cases of acute generalized exanthematous pustulosis (AGEP), nearly 20 cases of lichenoid drug eruptions of the skin or oral mucosa, vasculitis, pityriasis-rosea-like eruption, palmoplantar hyperkeratosis, and exacerbation of psoriasis. For mild to moderate rashes, antihistamines or, if necessary, topical or short course corticosteroids can be used. For severe skin reactions, the following management schedule has proved effective for achieving tolerance of therapeutic dosages of imatinib even after severe cutaneous reactions to the drug: prednisolone 1 mg/kg per day, tapered to 20 mg per day over several weeks along with the gradual reintroduction of imatinib (100 mg per day increased by 100 mg per week) given as the prednisolone dose is tapered off. This, of course, is only continued with if the skin manifestations do not recur. Oral desensitization to imatinib in patients with recurrent rash induced by the drug has been reported. Ten patients were subjected to a 4 h dosage procedure beginning with a dose of 10 ng followed by increases every 15 min. Four patients experienced no recurrence of rash after desensitization, four had recurrent rash that resolved after corticosteroid/antihistamine administration, and two patients each developed a rash and were unable to resume imatinib therapy.

13.3.3 Gefitinib and Erlotinib

Gefitinib and erlotinib are derivatives of 4-aminoquinazoline (Fig. 13.3). Both drugs are EGFR inhibitors, inhibiting the receptor's tyrosine kinase domain by binding to the ATP-binding site of the enzyme. Approved by the FDA in 2003, gefitinib is indicated for locally advanced or metastatic nonsmall cell lung cancer with activated mutations of EGFR tyrosine kinase. EGFR is over-expressed by the cells of some cancers such as lung and breast leading to uncontrolled cell proliferation, the blocking of apoptosis, and increased production of angiogenic factors and metastasis. The mutations also incur increased sensitivity to tyrosine kinase inhibitors like gefitinib, but no clinically beneficial activity of the drug is shown in patients with EGFR-negative tumors. The most frequent adverse reactions to gefitinib, that is reactions occurring in more than 20 % of patients, are diarrhea and skin reactions. Reactions may be categorized by the affected organ: skin reactions like hand-foot skin reaction, pruritus, erythema, and papulopustular rash are common as are nail disorders while bullous eruptions (erythema multiforme, SJS, and TEN) are rare. Note that hand-foot skin reaction should not be confused with hand-foot syndrome or acral erythema seen during the administration of some cytotoxic anticancer drugs such as 5-fluorouracil and doxorubicin. The former reaction is distinguished by localized blisters or hyperkeratosis whereas hand-foot syndrome shows diffuse, symmetrical erythematous, and edematous lesions on the palms and soles. Eye disorders (conjuctivitis, blepharitis), gastrointestinal disorders, vascular effects (hemorrhage), and renal and urinary disorders are also common. Interstitial lung disease has been found to occur in 1.3 % of patients, often of severe grade and occasionally fatal.

Erlotinib is an EGFR type 1 receptor (HER1/EFGR) tyrosine kinase inhibitor. These receptors are involved in the control of cell divisions and proliferation and by inhibiting their functions; erlotinib limits tumor cell division and metastasis and may even help in initiating apoptitic cell death.

A randomized, placebo-controlled, double-blind trial carried out by a National Cancer Institute of Canada Clinical Trials Group revealed that the main adverse responses to erlotinib were rash, fatigue, anorexia, diarrhea, nausea, ocular effects, infection, vomiting, and stomatitis. Rash and diarrhea were the main reasons for dose reduction and interruption of treatment. In a 2009 warning, the FDA referred to rare serious gastrointestinal, skin, and ocular disorders in some patients taking erlotinib. As with other tyrosine kinase inhibitors, papulopustular rash, hand-foot skin reaction, and pigmentary changes are commonly seen. Serious eye conditions include corneal lesions, some patients develop gastrointestinal perforations, and rare bullous and exfoliative skin reactions, some leading to death, have occurred.

13.4 Proteasome Inhibitors

13.4.1 The Proteasome

The ubiquitin-proteasome system has a central role in the turnover of proteins—in the regulation of cellular proteins involved in growth and survival and in the destruction of defective proteins. The proteasome consists of a hollow cylindrical or barrel-like 20S (0.7 MDa) proteolytic core capped at one or both ends by a 19S (0.9 MDa) regulatory particle or activator. These structures make up the single-capped proteasome complex or 30S double-capped form (Fig. 13.4). Note that the enzymically active double-capped proteasome complex, which is thought to be the functional unit in the cell, is usually referred to as the 26S (2.5 MDa) proteasome even though physicochemical analysis has revealed that the correct sedimentation coefficient is ~30S. The ~26S form probably represents the single-capped proteolytic core. Proteins that are defective in some way, for example, due to aging, incorrect folding, etc. are tagged by ubiquitin and directed to the proteasome for degradation via the endoplasmic reticulum degradation pathway. Cell-cycle progression is dependent on the ubiquitin-proteasome pathway with its three proteolytic activities in the proteasome that are mediated by three β-subunits in the core: β2 trypsin-like, β5 chymotrypsin-like, and β1 caspase-like activities. Proteolytic activity of each of these subunits is associated with the *N*-terminal threonine residues in peptide bond hydrolysis. Cancer cells show higher proteasome activity than normal cells. Inhibition of proteasome function leads to intracellular accumulation of unwanted proteins and ultimately cell death and, here too, cancer cells are more sensitive to the apoptosis-promoting effects of inhibition than normal cells. Proteasome inhibitors can induce apoptosis in leukemia- and lymphoma-derived cells without causing the death of non-transformed cells. Multiple myeloma cells synthesize and secrete large amounts of immunoglobulin, and this high rate of biosynthesis is thought to increase the sensitivity of the synthesized proteins to proteasome inhibitors by, for example, inducing the immunoglobulins into the unfolded state.

13.4.2 Bortezomib

Most proteasome inhibitors are short peptides that serve as protein substrates in the proteasome 20S core where they target the active site threonine residues. Bortezomib, an *N*-protected dipeptide that contains a boron atom (Fig. 13.5), was the first proteasome inhibitor to be introduced into the clinic and is approved for treating relapsed multiple myeloma and mantle cell lymphoma. The drug inhibits proteasomes by binding with high affinity via the boron atom to the β-subunit chymotrypsin- and caspase-like proteolytic catalytic sites. It has little effect on the trypsin-like activity. Apoptosis is normally suppressed in mantle cell lines and myeloma cells, but proteasome inhibition may overcome this suppression and activate cell death. Bortezomib suppresses tumor growth and spread and angiogenesis through multiple mechanisms and, in addition to directly inducing apoptosis of tumor cells, it mediates a myriad of biological effects including reduced adherence of myeloma cells to bone marrow cells, prevention of IL-6 production and signaling in myeloma cells, interference with

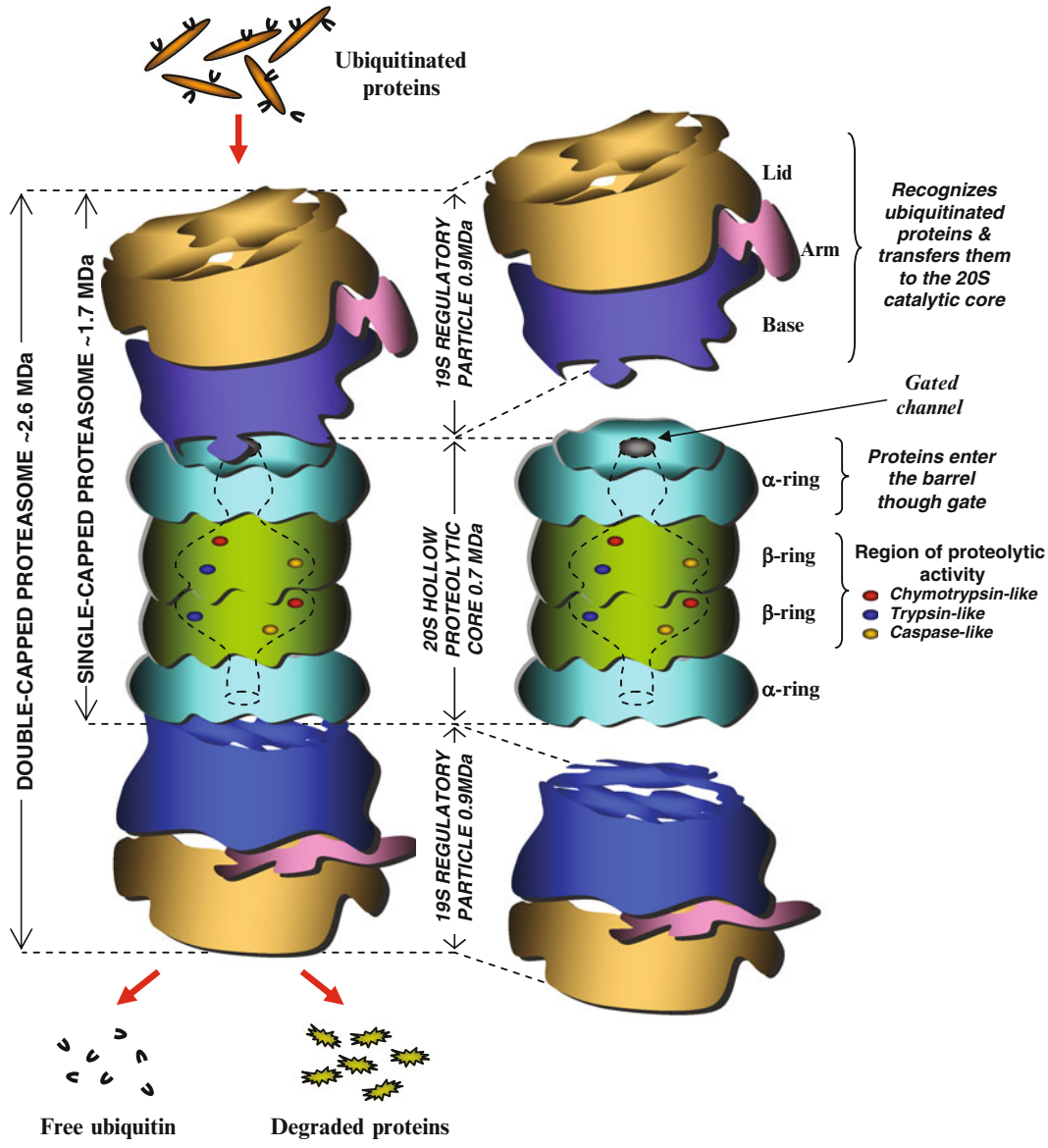

Fig. 13.4 Diagrammatic representation of the proteasome and its role in protein degradation via the ubiquitin-proteasome pathway. After being tagged with ubiquitin and unfolded for degradation on the 19S regulatory particles which aid the opening of a proteolytic gate in the 20S core, proteins are degraded into small peptides in the barrel-shaped core where β1 caspase-, β2 trypsin-, and β5 chymotrypsin-like activities reside. Regulatory particles are composed of a base (*dark blue*), lid (*yellow-brown*), and so-called arm (*pink*). The 20S proteolytic core may be capped at one or both ends by a 19S regulatory particle. Proteasomes are thus referred to as single-capped (sedimentation coefficient ~26S) or double-capped (~30S). In the literature, the term 26S proteasome is often used incorrectly when referring to the double-capped form. The double-capped complex is thought to be the functional proteasome unit in the cell

the production of pro-angiogenic factors, and suppression of nuclear-factor-κ-light chain-enhancer (NF-κB). Bortezomib gained regulatory approval in 2003, and its success has proved a stimulant in attempts to understand the molecular mechanisms underlying its clinical effectiveness and identifying new drugs acting on the same pathway.

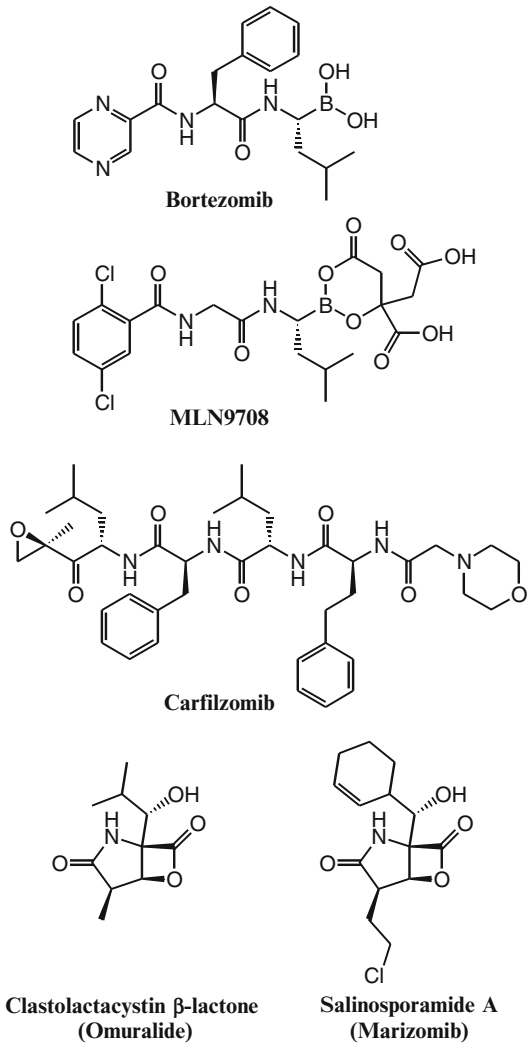

Fig. 13.5 Proteasome inhibitors used (or intended) for the treatment of relapsed multiple myeloma, mantle cell lymphoma, and some other tumors. Structures of the peptide boronates bortezomib and the orally active MLN9708, the peptide epoxyketone carfilzomib, and the γ-lactam-β-lactone bicyclics salinosporamide A (marizomib) and omuralide (β-clastolactacystin), both derived from natural sources. The drugs inhibit normal proteasome action by binding to the β-subunit proteolytic sites in the 20S core (see Fig. 13.4)

Gastrointestinal symptoms, thrombocytopenia, peripheral neuropathy, and neuropathic pain are the most common side effects of bortezomib and adverse cutaneous reactions to the drug are

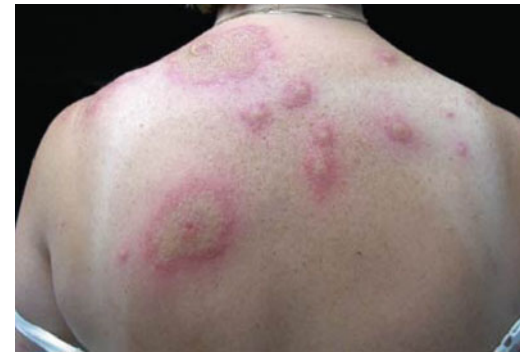

Fig. 13.6 An example of Sweet's syndrome, sometimes termed acute febrile neutrophilic dermatosis. The erythematous plaques that are characteristic of the condition and associated with fever and neutrophilic leukocytosis, are painful, and may occur on almost any part of the body. Most cases of Sweet's syndrome are idiopathic and although drugs rarely induce the reaction, besides bortezomib, at least 25 other drugs have been associated with the induction of the syndrome. (Reproduced with permission from Fam AG in Klippel JH, Stone JH, Crofford LeJ, White PH, editors. Primer on the Rheumatic Diseases, 13th ed. New York: Springer, 2008)

numerous. Rash (often pruritic) is frequently reported in more than 10 % of patients (an incidence of 8–18 % has been stated) and pruritus, erythema, urticaria, periorbital edema, and eczema are commonly seen. Bortezomib has been associated with a few cases of drug-induced Sweet's syndrome (Fig. 13.6) or acute febrile neutrophilic dermatosis, a rare variant of this uncommon skin disease characterized by fever, an elevated neutrophil count, and erythematous lesions infiltrated by neutrophils. Histological examination of a bortezomib-induced skin eruption showed a clinical picture similar to Sweet's syndrome but which differed by the presence of a significant number of CD30+ lymphocytes. The presence of these cells, which are seen during some treatments of blood malignancies, is not understood. Subcutaneous infusion of bortezomib as an alternative to intravenous administration was recently approved by the US Food and Drug Administration (FDA). This has proved a more convenient and less toxic route of administration and seems likely to become the standard form of the drug's delivery.

13.4.3 Second Generation Proteasome Inhibitors

Like bortezomib, *MLN9708* is also a peptide boronate (Fig. 13.5) but it is orally active, shows greater tissue penetration, and has a shorter half-life. The drug is primarily an inhibitor of the chymotrypsin-like activity of the 20S proteasome core and, like bortezomib, it inhibits NF-κB activation and has antitumor activity in multiple myeloma and some other hematologic malignancies. Besides peptide boronates like bortezomib, other synthetic compounds tested as proteasome inhibitors include peptide aldehydes, peptide epoxyketones, and peptide vinyl sulfones.

Carfilzomib, a peptide epoxyketone (Fig. 13.5) irreversibly binds to and inhibits chymotryptase activity but has less activity toward the other two ezymatic actions. The drug was approved by the FDA in 2012 for patients with relapsed and refractory multiple myeloma. It leads to cell cycle arrest and induces apoptosis in multiple myeloma, other hematologic malignancies, and some solid tumors. A potentially very important property is the drug's activity against primary multiple myeloma cells and cell lines resistant to bortezomib.

Adverse reactions noted so far include pulmonary hypertension, dyspnea, cardiac disorders, cytopenias, infusion reactions, tumor lysis syndrome, hepatotoxicity, peripheral neuropathy, rash, and urticaria.

The proteasome inhibitors *salinosporamide A* and *omuralide* are both γ-lactam-β-lactone bicyclic compounds (Fig. 13.5) derived from natural sources. Salinosporamide A, also known as *marizomib*, is obtained from *Salinospora tropica,* a bacterium found in ocean sediments. The drug is an irreversible proteasome inhibitor that shows little effect on the caspase-like activity but inhibits chymotrypsin- and trypsin-like protease activities. Preclinical studies have demonstrated antitumor activity in models for multiple myeloma, hematologic malignancies, and solid tumors and, importantly, marizomib does not show cross-resistance with other proteasome inhibitors. Omuralide (clasto-lactacystin β-lactone; β-clastolactacystin) is the active metabolite of lactacystin isolated from *Streptomyces spp.* Lactacystin inhibits trypsin- and chymotrypsin-like activity of the 20S proteasome. Omuralide inhibits cell cycle progression in several tumor cell lines.

13.5 Cytokine-release and tumor lysis syndromes

Cytokine-release syndrome (CRS), also called acute infusion reaction, is not a true hypersensitivity reaction but the two share some signs and symptoms such as nausea, fever, cough, dyspnea, bronchospasm, hypotension, rash, itching, and urticaria. CRS is usually short term, developing during or soon after drug infusion followed by resolution within 24 hours. Drug-induced destruction of cells is thought to release cytokines such as TNF and interleukins producing symptoms similar to type I hypersensitivity.

Within 48-72 hours of initiating cancer therapy, large numbers of tumor cells may be destroyed in a short time releasing intracellular contents and producing ionic imbalances in calcium, phosphate, potassium, and uric acid. This condition, termed tumor lysis syndrome (TLS), may progress to acute renal failure, cardiac arrhythmias, seizures, and death. Unlike CRS, TLS is easy to distinguish from drug-induced hypersensitivity. Drugs causing TLS include etoposide, fludarabine, hydroxyurea, paclitaxel, thalidomide, and bortezomib.

Summary

- In excess of 100 drugs are currently administered in significant quantities to cancer patients somewhere in the world. Many of the drugs used have been, and still are, alkylating agents, antimetabolites, organoplatinum compounds, cytoskeletal disruptors, or anthracyclines, all agents with relatively broad rather than targeted and specific modes of action.
- A more refined strategy of specific action on a particular cancer cell rather than nonspecific inhibition and killing has been used in

developing some of the more recently introduced agents. The tyrosine kinase inhibitor imatinib mesylate and proteasome inhibitor bortezomib are examples of the more specific approach.

- Taxanes such as docetaxel and paclitaxel are mitotic inhibitors, disrupting microtubule function so adverse reactions, including hypersensitivity responses, to these drugs might be expected.
- Up to 30 % of patients develop acute infusion reactions to taxanes. Acute hypersensitivity reactions are marked by urticaria, flushing, rashes, dyspnea, gastrointestinal symptoms, hypo- and hypertension, and back pain.
- Patients with severe hypersensitivity to paclitaxel also react to docetaxel, giving a cross-sensitivity rate of ~90 %.
- Premedication regimen for docetaxel: oral dexamethasone 8 mg twice daily for 3 days starting 24 h prior to the commencement of infusion. For paclitaxel infused over 1, 3, and 24 h: H_1 antagonist diphenhydramine (50 mg) and an H_2 antagonist (cimetidine 300 mg, ranitidine 50 mg, or famotidine 20 mg) given IV prior to infusion beginning. Oral dexamethasone 20 mg administered 12 and 6 h prior.
- Up to 50–70 % of cancer patients are treated with platinum drugs. Treatment with platinum drugs is often effected in combination with other anticancer agents.
- Mild reactions to platinum drugs are rash, urticaria, flushing, palmar itching, a burning feeling, hand and facial edema, pruritus, back pain, abdominal cramping, and diarrhea. Moderate to severe symptoms include diffuse erythroderma, tachycardia, chest tightness, wheezing, facial swelling, dyspnea, hypertension, or hypotension. Severe symptoms include bronchospasm, chest pain, seizures, and systemic anaphylaxis that may be life-threatening.
- Most reactions to platinum drugs appear after multiple treatment cycles (usually at least six).
- Reactions to the platinum drugs are mainly type I or type IV hypersensitivity responses with a few cases of type II and type III hypersensitivities.
- Skin testing with the three platinum drugs has been employed to identify at-risk patients and predict platinum hypersensitivity. As yet, skin testing with the drugs has not found widespread acceptance and application as a routine diagnostic procedure.
- A 12 step, ~ 6 h desensitization protocol for carboplatin has been successfully employed in skin test-positive patients hypersensitive to the drug.
- The Philadelphia translocation t(9;22)(q34;q11) or Philadelphia chromosome is a chromosomal defect resulting in gene fusion of the BCR and ABL genes. The resultant fusion gene is the BCR-ABL oncogene. The Philadelphia chromosome is a cytogenetic abnormality seen in 95 % of chronic myeloid leukemia patients and 15–30 % of adults with acute lymphoblastic leukemia.
- The drug imatinib mesylate that inhibits both the ABL and BCR-ABL tyrosine kinases has been successful in treating chronic myeloid leukemia.
- In addition to imatinib, some inhibitors of receptor tyrosine kinases targeting epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptors (VEGFR) and platelet-derived growth factor receptors (PDGFR) have been found to have antitumor and/or other activities.
- For most of the tyrosine kinase inhibitors targeting EGFRs, papulopustular rash; hand-foot skin reaction; pigmentary changes; xerosis; pruritus and mouth, hair, scalp, and nail abnormalities are the primary adverse events.
- Imatinib is generally well tolerated especially when compared to most cytotoxic chemotherapies. The most common non-hematologic adverse reactions include superficial edema, nausea, diarrhea, muscle cramps, and vomiting and, most common of all, cutaneous reactions. Patients receiving standard dose imatinib therapy in the chronic phase of chronic myeloid leukemia experience neutropenia in 35–45 % of cases, thrombocytopenia in 20 %, and anemia in 10 % of cases.
- Although most cutaneous reactions to imatinib are mild and dose dependent, severe

reactions such as SJS, exfoliative dermatitis, TEN, AGEP, DRESS, and lichenoid eruptions have been reported.

- Gefitinib and erlotinib are EGFR inhibitors, inhibiting the receptor's tyrosine kinase domain by binding to the ATP-binding site of the enzyme. EGFR is over-expressed by the cells of some cancers such as lung and breast leading to uncontrolled cell proliferation.
- The most frequent adverse reactions to gefitinib are diarrhea and skin reactions. The main adverse responses to erlotinib are rash, fatigue, anorexia, diarrhea, nausea, ocular effects, infection, vomiting, and stomatitis. The FDA has referred to rare serious gastrointestinal, skin, and ocular disorders in some patients taking erlotinib.
- Bortezomib, an *N*-protected dipeptide that contains a boron atom, inhibits proteasomes by binding via the boron atom to the catalytic site of the 26S proteasome with high affinity. This ultimately leads to the killing of multiple myeloma cells.
- Gastrointestinal symptoms, thrombocytopenia, peripheral neuropathy, and neuropathic pain are the most common side effects of bortezomib, and adverse cutaneous reactions to the drug are numerous—rash is frequently reported in more than 10 % of patients.
- Newer proteasome inhibitors include the orally active peptide boronate MLN9708 and carfilzomib. Adverse reactions to carfilzomib include pulmonary hypertension, dyspnea, infusion reactions, tumor lysis syndrome, hepatotoxicity, rash, and urticaria.

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Proton Pump Inhibitors 14

Abstract

Proton pump inhibitors (PPIs), omeprazole, lansoprazole, pantoprazole, rabeprazole, esomeprazole, and dexlansoprazole bind irreversibly to the H^+, K^+- ATPase (the "proton pump") inhibiting its activity and decreasing gastric acid production. Systemic reactions to PPIs include anaphylaxis, urticaria, angioedema, interstitial nephritis, and thrombocytopenia. Cutaneous reactions include contact dermatitis, maculopapular and lichenoid eruptions, vasculitis, exfoliative erythrodermia, AGEP, DRESS, and SJS/TEN. Autoimmune reactions, including cutaneous lupus erythematosus, have been described. Cross-reactions between PPIs may be limited to one or two drugs or all drugs may be recognized. Cross-reaction studies so far have been based on skin testing, but the interpretations lack a quantitative basis. Successful oral desensitization following anaphylaxis to a PPI has been achieved in a few hours. Skin testing and challenge testing have been the only procedures employed to diagnose immediate reactions to PPIs. A suitable test for the detection of PPI-specific IgE antibodies is not yet available, and application of the positive basophil activation test has been limited.

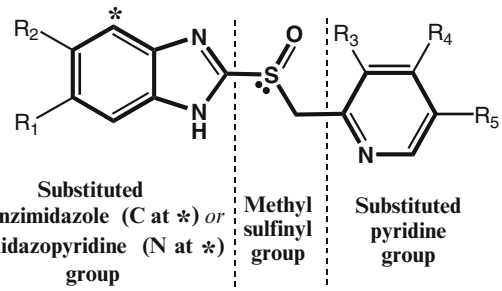
Proton pump inhibitors (PPIs) reduce gastric acid production in a pronounced and sustained manner. They are the most potent of the drugs that inhibit gastric acid secretion and are now widely used, essentially replacing the formerly heavily used histamine H_2-receptor antagonists.

14.1 Chemistry

All marketed PPIs, omeprazole, lansoprazole, pantoprazole, rabeprazole, esomeprazole, and dexlansoprazole are benzimidazole derivatives with the timoprazole backbone structure (Table 14.1). Esomeprazole is the *S*-enantiomer of omeprazole and dexlansoprazole the *R*-enantiomer of lansoprazole. The structures of each of these PPIs consist of substituted pyridine and a benzimidazole heterocyclic center linked by a methylsulfinyl group. Some new PPIs being developed, for example, tenatoprazole (Table 14.1), have an imidazopyridine instead of the benzimidazole ring structure. The imidazopyridine drugs have a longer half-life than the existing PPIs.

B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2_14, © Springer Science+Business Media, LLC 2013

Table 14.1 Chemical structures of benzimidazole proton pump inhibitors (PPIs) showing the timoprazole backbone structure and structure of tenatoprazole, a new generation imidazopyridine PPI



General structure of PPI

Proton pump inhibitor (PPI)	Atom at pos. *	Enantiomorph at *S* sulfinyl	$-R_1$	$-R_2$	$-R_3$	$-R_4$	$-R_5$
Backbone structure							
Timoprazole	C	–	–H	–H	–H	–H	–H
Benzimidazole group							
Omeprazole[a]	C	*RS*[a]	$-OCH_3$	–H	$-CH_3$	$-OCH_3$	$-CH_3$
Esomeprazole[b]	C	*S*[b]	$-OCH_3$	–H	$-CH_3$	$-OCH_3$	$-CH_3$
Lansoprazole[c]	C	*RS*[c]	–H	–H	$-CH_3$	$-OCH_2CF_3$	–H
Dexlansoprazole[d]	C	*R*[d]	–H	–H	$-CH_3$	$-OCH_2CF_3$	–H
Rabeprazole	C	*RS*	–H	–H	$-CH_3$	$-O(CH_2)_3OCH_3$	–H
Pantoprazole	C	*RS*	$-OCHF_2$	–H	$-OCH_3$	$-OCH_3$	–H
Imidazopyridine group							
Tenatoprazole	N	*RS*	–H	$-OCH_3$	$-CH_3$	$-OCH_3$	$-CH_3$

[a]Omeprazole is a 1:1 racemic mixture of the *R*- and *S*-enantiomers
[b]Esomeprazole is the *S*-enantiomer of omeprazole
[c]Lansoprazole is a 1:1 racemic mixture of the *R*- and *S*-enantiomers
[d]Dexlansoprazole is the *R*-enantiomer of lansoprazole

14.2 Mechanism of Action

The PPIs are prodrugs, activated by exposure to pHs less than 5. Once activated, the drugs bind irreversibly to the H^+, K^+- ATPase (the "proton pump") in the parietal cell apical membrane, inhibiting its activity and decreasing gastric acid production by more than 95 %. The process is irreversible in that new enzyme needs to be produced to overcome the inhibition. PPIs have little effect on gastric acid volume and do not affect gastric motility.

14.3 Hypersensitivity Reactions to Proton Pump Inhibitors

Hypersensitivity reactions to PPIs may be mild but the spectrum of possible reactions is wide and some may be severe and life-threatening. Systemic reactions include anaphylaxis, urticaria, angioedema, acute interstitial nephritis, cytopenia, thrombocytopenia, and vasculitis. Cutaneous reactions include occupational contact dermatitis, photoallergic dermatitis, lichenoid eruption, erythema nodosum, pytiriasis rosea, exfoliative erythrodermia, acute generalized exanthematous pustulosis (AGEP), fixed drug eruption, maculopapular eruption, drug reaction with eosinophilia and systemic symptoms (DRESS), and Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN). Autoimmune reactions, including cutaneous lupus erythematosus, have been described.

There are a number of reports of anaphylaxis to PPIs, particularly omeprazole and pantoprazole. This may reflect usage. Recent figures on the incidence of anaphylaxis to PPIs are hard to find but as of May 1999, the Uppsala Monitoring Centre database contained 42 reports of anaphy-

lactic reactions to the drugs with omeprazole, lansoprazole, and pantoprazole showing incidences (percentages of all reported adverse reactions) of 0.2, 0.2, and 0.4 %, respectively. Judging by the number of reports in the literature, it seems certain that the number of cases of anaphylaxis to PPIs since 1999 is considerably more than 42. Investigations of immediate reactions to PPIs have generally been carried out by skin testing, sometimes yielding results that provide information on cross-recognition between the different drugs as well as the drug(s) provoking the reaction. In one example, a patient with a severe immediate reaction to lansoprazole confirmed by skin prick testing and challenge with the drug also reacted to a 5 mg challenge with rabeprazole, despite showing negative skin reactions to that drug, omeprazole, and pantoprazole and negative challenge tests to omeprazole and pantoprazole. Possible cross-reactivity between lansoprazole and rabeprazole was also demonstrated in a separate study by intradermal tests on a patient allergic to the former drug. Investigations of immediate reactions to PPIs have revealed allergic recognition of omeprazole and lansoprazole in the same patient, and one case of hypersensitivity to omeprazole showed a prick test-positive response to lansoprazole. Other observed patterns of limited cross-reactivity include recognition of omeprazole, lansoprazole, and pantoprazole and cross-recognition between omeprazole and pantoprazole. There is also a report of a patient with anaphylaxis and a positive skin test to omeprazole and a negative skin test to pantoprazole, lansoprazole, esomeprazole, and rabeprazole. Another case report describes a patient allergic to omeprazole but tolerant to both pantoprazole and lansoprazole (esomeprazole and rabeprazole were not tested). The clinical features of immediate reactions to PPIs suggest an IgE antibody-mediated mechanism and the observed cross-reactions likely reflect antibody cross-recognition of fine structural features on the different drugs. Omeprazole and pantoprazole are structurally fairly similar; the former has a methoxy substituent on the benzimidazole group while the latter has a difluoromethoxy group at the equivalent position and an extra methoxy on the pyridine ring. Lansoprazole and rabeprazole differ only at position 4 on the pyridine ring where the former has a trifluoroethoxy and the latter a methoxypropoxy group (Table 14.1). It seems likely that structures of the two drugs are sufficiently similar to be recognized by some IgE antibodies. While these findings demonstrate limited cross-reactivities, other investigations have detected cross-reactivity covering all of the PPIs in current use. This area of PPI hypersensitivity research has essentially been based on clinical studies principally using skin testing to demonstrate cross-recognitions, and the interpretations lack a quantitative basis. Application of quantitative hapten inhibition experiments alongside skin test results are sorely needed, but this will be difficult without a suitable method for the detection of PPI-reactive IgE antibodies in allergic patients' sera.

Successful oral desensitization of a patient who experienced anaphylaxis to omeprazole was achieved after 5.6 h, starting with an initial dose of 1 μg of drug and ending with a full dose of 16 mg for a total cumulative dose of 32.6 mg. After the desensitization, the patient was able to tolerate the full dose uneventfully and the wheal size of the intradermal response to omeprazole was significantly reduced.

There appears to be fewer reports of delayed reactions to PPIs, but the range of adverse skin reactions seen is wide. Pantoprazole, for example, has been implicated in severe cutaneous responses including SJS/TEN, lichenoid eruption, exfoliative erythrodermia, and vasculitis. At least one fatal reaction has occurred following TEN induced by a PPI. Maculopapular eruptions and pruritus are frequently seen and mild in intensity. Erythrodermic reactions to omeprazole and lansoprazole and allergic contact dermatitis to lansoprazole have also been reported. A case of DRESS induced by esomeprazole is noteworthy since it involved co-sensitivity to other PPIs and suggested caution in skin testing PPIs in patients with severe reactions. Patch testing using esomeprazole as a 10 % solution gave a positive reaction at 48 and 72 h. A second series of patch tests proved positive to omeprazole and pantoprazole as well as to esomeprazole, but no

reaction was seen with rabeprazole. Histological examination of the esomeprazole-positive test showed typical signs of a delayed hypersensitivity response. At 60 h after the second tests, the patient experienced a mild erythroderma with facial edema and desquamation, indicating induction of a flare of DRESS.

14.4 Diagnosis of Hypersensitivity to Proton Pump Inhibitors

Skin testing and to a lesser extent challenge testing have almost invariably been the only clinical or laboratory test procedures employed in the diagnosis of immediate reactions to PPIs. Prick test concentrations used have shown up to a tenfold variation: for omeprazole and pantoprazole, 4–40 mg/ml; esomeprazole, 20–40 mg/ml; lansoprazole, 3–30 mg/ml; and rabeprazole, 10–20 mg/ml. Solutions of omeprazole and pantoprazole have been prepared by dissolving lyophilized drug in physiological saline while solutions of the other three PPIs are usually formulated from crushed and powdered tablets. Reported investigations have not always included results of skin tests on nonallergic controls. For intradermal testing, the concentration ranges used have been more consistent—omeprazole and pantoprazole, 0.04–8 mg/ml; lansoprazole, 0.015–3 mg/ml; esomeprazole, 0.02–2 mg/ml; rabeprazole, 0.01–2 mg/ml. These concentrations generally represent 1:10, 1:100, and 1:1,000 serial dilutions of the prick test concentrations with testing starting at the lowest concentration and stepping up until a positive reaction results. So far, there is limited information available on patch testing with PPIs. Test concentrations employed are in the range 10–30 % in petrolatum or aqueous medium, and tests are generally read at least twice after 48–96 h. If cutaneous reactions are severe, great caution should be exercised.

Oral challenge with lansoprazole of a patient who experienced an anaphylactic-type reaction to the drug provides an example of the use of this test in confirming a diagnosis of an immediate reaction to a PPI. Three doses of lansoprazole, 7.5, 15, and 30 mg, were given at 60 min intervals. Twenty minutes after the third and final dose, that is after a total dose of 52.5 mg, the patient reacted with erythema of palms, itching, rash, and malaise.

A recently published (2012) European multicenter study compared the diagnostic accuracy of skin and oral provocation tests in patients with immediate hypersensitivity to PPIs. Patients with reactions that were not immediate were excluded. Skin prick tests were performed with solutions of omeprazole, esomeprazole, pantoprazole, and rabeprazole at 40 mg/ml and lansoprazole, 30 mg/ml. Omeprazole, esomeprazole, and pantoprazole were used in intradermal tests at 0.4 and 4 mg/ml. Oral provocation tests carried out on some patients after skin testing consisted of the administration of four talc capsules on day one followed on day two by lansoprazole (5, 10, 15 mg) or one of the other four drugs (5, 5, 10, 20 mg) at 30 min intervals. Skin tests were positive in 12 of 53 patients; four of these underwent provocation testing with the suspected PPI and in each case a positive response was obtained. Provocation tests on the 41 patients with a negative skin test showed three more positive reactors. For the skin tests, specificity and the positive predictive value were both 100 %. The negative predictive value was 91.9 %. A higher frequency of skin test positivity occurred in patients with severe reactions and cross-reactions consistent with previous observations were observed. The study's authors concluded that skin testing with PPIs on patients with immediate hypersensitivity to these drugs is a useful diagnostic test, and the test has the additional advantage of allowing the clinician to avoid oral challenges.

So far a suitable test for the detection of PPI-specific IgE antibodies does not appear to be available. There are at least two reports of a positive basophil activation test on patients allergic to omeprazole—one utilizing the CD63 basophil marker that was positive to omeprazole but negative to pantoprazole and the other detected by the flow-cytometric cellular allergen simulation test (FAST).

14.5 Proton Pump Inhibitors, Gastroesophageal Reflux Disease, and Asthma

There appears to be a higher incidence of asthma in children with gastroesophageal reflux disease (prevalence estimated to be 34–89 %), and this has prompted the suggestion, and the belief seemingly supported by some studies, that PPI treatment of these children may lead to an improvement in asthma symptoms. Three randomized trials showed that PPIs had a beneficial effect on asthma symptoms but one randomized, double-blind, placebo-controlled trial failed to show that omeprazole improved symptoms. At present, there is not enough data from well constructed and controlled clinical trials to reach a confident and conclusive decision on this question.

14.6 Other Safety Concerns with Proton Pump Inhibitors

Besides hypersensitivity responses to PPIs, there are at least four other specific concerns related to the interactions and/or direct effects of PPIs in humans. The oral antiplatelet drug clopidogrel is used to inhibit blood clots. PPIs inhibit the bioactivation of clopidogrel to its active metabolite and reduce the antiplatelet effects of the drug. It has been suggested that this may lead to an increased risk of vascular events. The results of a recent randomized control trial with clopidogrel and omeprazole do not add support to this belief, but the makers of PPIs have agreed to work with the FDA to conduct studies to obtain additional information that will allow a better understanding of the effects of PPIs on clopidogrel. A second concern associated with PPIs is a suggested link between the drugs and fractures. Some believe that this could be related to altered absorption of calcium, vitamin B_{12}, or iron. Clear evidence to support an association with bone fractures is, at present, lacking and no convincing mechanism has been suggested, so the alleged association remains to be resolved. Thirdly, the possibility that long-term PPI use might lead to hypomagnesemia has led the FDA to suggest that serum magnesium levels of patients taking PPIs should be monitored. Again, the mechanism of such an effect of the PPIs is unclear. More clinical data are needed in the case of each of these three concerns and until that is the situation, clinicians and researchers should remain aware and keep abreast of developments in each area. Lastly, the use of PPIs has been shown to be a significant risk for both community- and hospital-acquired pneumonia.

Summary

- All marketed PPIs, omeprazole, lansoprazole, pantoprazole, rabeprazole, esomeprazole, and dexlansoprazole are benzimidazole derivatives. Some new PPIs being developed have an imidazopyridine instead of the benzimidazole ring structure.
- The PPIs are prodrugs, activated by exposure to pHs less than five. Once activated, the drugs bind irreversibly to the H^+, K^+- ATPase (the "proton pump") in the parietal cell apical membrane, inhibiting its activity and decreasing gastric acid production by more than 95 %.
- Systemic reactions to PPIs include anaphylaxis, urticaria, angioedema, interstitial nephritis, cytopenia, thrombocytopenia, and vasculitis.
- Cutaneous reactions include occupational contact dermatitis, photoallergic dermatitis, pruritus, maculopapular eruptions, vasculitis, lichenoid eruption, erythema nodosum, pityriasis rosea, exfoliative erythrodermia, AGEP, fixed drug eruption, DRESS, and SJS/TEN. Autoimmune reactions, including cutaneous lupus erythematosus, have been described.
- Patterns of limited cross-reactivity include recognition of omeprazole, lansoprazole, and pantoprazole and cross-recognition between omeprazole and pantoprazole. Other investigations have detected cross-reactivity covering all of the PPIs in current use.
- Cross-reaction studies so far have been based on skin testing, and the interpretations lack a

quantitative basis. Development of IgE tests and application of quantitative hapten inhibition experiments alongside skin test results are needed.

- Successful oral desensitization of a patient who experienced anaphylaxis to omeprazole was achieved after 5.6 h.
- Skin testing and to a lesser extent challenge testing have almost invariably been the only clinical or laboratory test procedures employed in the diagnosis of immediate reactions to PPIs.
- A recently published multicenter study compared the diagnostic accuracy of skin and oral provocation tests in patients with immediate hypersensitivity to PPIs. For the skin tests, specificity and the positive predictive value were both 100 %. The negative predictive value was 91.9 %.
- A suitable test for the detection of PPI-specific IgE antibodies is not yet available, and application of the positive basophil activation test has been limited.
- Other safety concerns with PPIs include the suggested inhibition of the antiplatelet effects of clopidogrel leading to an increased risk of vascular events, and associations with bone fractures, hypomagnesemia, and pneumonia.

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Postface – Concluding Remarks

Drug Allergy Research in the Immediate Future: The Promise of Elucidating Mechanisms of the Immune Response to Small Molecules

Work on, and progress in, answering outstanding questions in drug allergy today has the potential not only to contribute significantly to the diagnosis and treatment of poorly understood, difficult to manage, and sometimes life-threatening hypersensitivities but also to provide insights into some of the most fundamental and important areas of immunological investigation. These include cellular recognition and interactive processes, relationships between receptors and effector and signaling pathways, mechanisms of mediator action, and the genetic basis of drug reactions. More specifically, insights into the mechanisms of hypersensitivity and some other intolerant reactions to drugs are likely to expand knowledge of and/or lead to:

- The immune response's capacity (both humoral and cellular) to distinguish fine structural differences on structurally closely related "small" molecules.
- Which drugs are recognized as hapten–protein complexes requiring processing by antigen-presenting cells and which drugs activate an immune response without covalent binding. In each case, the mechanisms involved are likely to be amenable to investigation and have the prospect of offering fascinating insights into the allergic recognition of different structures.
- Improved phenotyping of patients to clearly identify associations with individual drug-induced skin hypersensitivities.
- HLA–drug hypersensitivity associations (as already seen, for example, with carbamazepine, allopurinol, and abacavir). Should prior genotyping be employed for some drugs? The fascinating recent demonstration of modification by abacavir of the HLA–peptide repertoire and its effect on immune self-reactivity already promises to add a new dimension to the way we look at, and seek to understand, autoimmunity.
- The relationship between cutaneous and systemic reactions in diseases such as drug reaction with eosinophilia and systemic symptoms (DRESS or hypersensitivity syndrome) and why the same drug produces both reactions in some patients but only cutaneous reactions in others.
- Insights into cellular recognition of antigens in the skin, resultant cell interactions, and subsequent cell-mediated toxic effects.
- Identification and mechanisms of action of important toxic mediators of cell damage in local reactions.
- Further elucidation of drug receptor (histamine, leukotriene, PAF, etc.)-activated signaling pathways.
- Implication of the involvement of new mediators in drug-induced anaphylaxis and cutaneous reactions.
- Possible different pathways of anaphylaxis in humans.
- The in vivo cellular effects and clinical consequences of sudden and complete removal of the

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culprit drug in allergic patients by selective sequestration as seen, for example, with sugammadex in anaphylaxis induced by the neuromuscular blocker rocuronium.
- The origin of preexisting allergic sensitivity in patients who react to a drug without previous exposure.
- Detailed mechanisms involved in drug-induced redirection of synthetic metabolic pathways thought to be operative, for example, in sensitivities to nonsteroidal anti-inflammatory drugs.
- The interrelationship between the anti-inflammatory and immunosuppressive actions of corticosteroids on the one hand and, on the other, the body's allergic responses to the drugs.
- The molecular basis of cutaneous and systemic allergic cross-recognition of corticosteroids.
- Genetic engineering advances in improving the selectivity of therapeutic monoclonal antibodies (mAbs) in terms of their improved pharmacokinetics, binding affinities, specificities, toxicities, and increased half-life.
- The development of more, and improved, recombinant proteins (like, for example, etanercept and denileukin diftitox), specifically targeted at selected ligands or receptors involved in disease.
- The development of novel and therapeutically more effective inhibitors of the ubiquitin–proteasome pathway for chemotherapy of cancer cells.
- Development of improved forms of drug delivery, dosage schedules, and optimum routes of administration for some important drugs known to cause infusion and other severe reactions but not able to be substituted (e.g., some specific anticancer drugs).
- Development of effective desensitization procedures for important and increasingly used biologic agents and anticancer drugs commonly provoking hypersensitivity and other adverse reactions.

Progress varies in the continuing efforts to understand different mechanisms in the above-listed, far from exhaustive, summary of important research categories. Each is a major and often difficult research area in itself, but, in every case, relatively recent insights have advanced to the stage where increasingly relevant questions can be asked with the expectation that further significant progress will result in the not-too-distant future. As mentioned immediately above and discussed earlier in this book, the recent elucidation of the mechanism underlying abacavir-induced hypersensitivity looks likely to prove a turning point in the way we look at small molecules and the immune response and the possible effects drugs, environmental toxins, and other chemicals might have in altering T cell immune responses. After being overlooked for so long on the fringe of mainstream immunology and virtually ignored in its textbooks, drug allergy research now appears to offer paths that might lead to previously unsuspected advances in understanding mechanisms of diseases where the interrelationship between genetics, biochemical interactions, and immune recognition processes had previously appeared too daunting in terms of both the biological complexities and the seemingly illogical nature of the drug-induced outcomes. For example, how could small amounts of some chemically unrelated drugs, often apparently chemically unreactive, provoke a catastrophic cutaneous and systemic reaction such as toxic epidermal necrolysis or Stevens–Johnson syndrome or DRESS or a range of other severe reactions that seem to have an immune basis? Could it turn out, as already suggested, that research into the immunopathogenesis of such poorly understood drug-induced hypersensitivities also leads us to a better understanding of autoimmune disease and perhaps even infectious diseases and cancer?

Finally, at this exciting time in the expansion of knowledge of the many known and suspected drug allergies and intolerances, it seems important to keep a realistic *perspective* of the everyday medical importance of reactions to different drugs or groups of drugs. Such a perspective should relate to the frequencies and severities of reactions, short- and long-term morbidities, mortalities, economic costs, and the consequences for continuing drug therapy with the offending drug and/or other drugs. A quick perusal of this volume reveals that what have

long been regarded by many as "true" allergies (e.g., immediate reactions such as drug-induced urticaria/angioedema/anaphylaxis, many drug-induced exanthematous reactions such as maculopapular exanthema, allergic contact dermatitis) still constitute a large proportion of allergic drug reactions throughout the world, and this is even more apparent if the whole spectrum of "hypersensitivities" to the nonsteroidal anti-inflammatory drugs is included. Indeed, significant increases in the incidences of both urticaria and anaphylaxis to drugs have recently been reported in a number of countries. However, in recent years, the allergy literature at times gives the impression that drug-induced delayed hypersensitivity reactions constitute almost the sole area of clinical and research interest in drug allergy and for patient importance. There is no doubting the advances made and the severe consequences for patients of some drug-induced delayed reactions, but it should be remembered that a high proportion of patients with rashes and urticaria-like reactions are eventually shown to tolerate the previously suspected drug and many of the severe responses remain extremely rare events. While research on reactions to drugs such as sulfamethoxazole, carbamazepine, allopurinol, and abacavir has yielded important scientific and clinical insights and provided knowledge that may be useful in preventing these and other potential drug toxicities, the numbers of treated patients are often relatively small and the drug reactions sometimes predominate in often small groups of patients where special circumstances are involved. For example, apart from the treatment of HIV patients, drugs such as sulfamethoxazole and abacavir are not widely and heavily used and cases of drug-induced severe reactions like erytheme multiforme, Stevens–Johnson syndrome, and toxic epidermal necrolysis are infrequent with collective incidences of all three estimated to be 7, 1.8, and 9 per 10^6 person-years for patients <20, 20–64, and 65 years of age and older, respectively. For toxic epidermal necrolysis alone, the overall incidence of hospitalization is ~0.5 per 10^6 person–years. By comparison, urticaria (the second most common drug-induced cutaneous reaction after erythematous reactions) and anaphylaxis, for example, are far more commonly encountered and there is still much to understand about both conditions. Comparing the above frequencies for the severe, toxic bullous and other delayed cutaneous reactions to those for the most common offending drugs causing adverse drug reactions (both true hypersensitivities and drug intolerances), namely, nonsteroidal anti-inflammatory drugs, the penicillins, cephalosporins, other antibiotics, the numerous drugs used in anesthesia and surgery, therapeutic mAbs, antineoplastic drugs, contrast media, and opioid analgesics, provides a truer perspective of drug "allergy" than that gleaned from many current research priorities.

In summary, while there is, and should be, a research emphasis on the still poorly understood aspects of a number of cutaneous and cutaneous/systemic drug-induced syndromes, and the considerable benefits of this research both at the clinical and fundamental scientific levels are likely to extend beyond the confines of drug allergy, the majority of "allergic" drug reactions in everyday clinical practice usually fall outside the currently favored areas of the most intense research interest. In this volume we have attempted to cover the full range of different drug hypersensitivities and intolerances, from common hives to life-threatening anaphylaxis and simple erythematous rash to toxic epidermal necrolysis, with the aim of providing information on the immune and some nonimmune drug reactions and their diagnoses. In addition, and with clinicians, researchers, teachers, and students in mind, efforts have been made to present a critical but balanced perspective of the importance and incidences of reactions, our current understanding of mechanisms underlying the various drug hypersensitivities and intolerances, the remaining important gaps in our knowledge, and some likely important areas for future research.

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B.A. Baldo and N.H. Pham, *Drug Allergy: Clinical Aspects, Diagnosis, Mechanisms, Structure-Activity Relationships*, DOI 10.1007/978-1-4614-7261-2, © Springer Science+Business Media, LLC 2013

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