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Drug Allergy (C Mayorga, Section Editor)

New Insights of Biomarkers in IgE and Non-IgE-Mediated Drug Hypersensitivity

Cristobalina Mayorga, PhD[1,2,3,*]
R. Muñoz-Cano, MD, PhD[4,5]
A. Rodríguez-Nogales, PhD[1]
R. Fernandez-Santamaría, BsC[1]
T. D. Fernandez, PhD[1]

Address
[1]Allergy Research Group, Instituto de Investigación Biomédica de Málaga-IBIMA. ARADyAL. Hospital Civil, 29009, Málaga, Spain
[2]Allergy Unit, Hospital Regional Universitario de Málaga. ARADyAL Hospital Civil, 29009, Málaga, Spain
[*,3]Nanostructures for Diagnosing and Treatment of Allergic Diseases Laboratory, Andalusian Center for Nanomedicine and Biotechnology-BIONAND, Málaga, Spain
Email: lina.mayorga@ibima.eu
[4]Allergy Section, Neumology Department, Hospital Clinic, ARADyAL, University of Barcelona, Barcelona, Spain
[5]Clinical and Experimental Respiratory Immunoallergy (IRCE), August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain

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Abstract

Background Drug hypersensitivity reactions (DHRs) occurring less than 6 h after the drug intake are named immediate reactions. They include allergic reactions, and pseudo-allergic or non-allergic reactions, and despite their similar clinical manifestations, the underlying mechanism is different. Its identification is essential for their management. In IgE-mediated-DHRs, the best biomarker is drug-specific IgE, which can be determined by in vivo and in vitro tests. Identifying the culprit drug is critical for the design of avoidance strategies and the recommendation of safe alternatives for future treatments.
Recent findings It has been suggested the existence of other mechanisms beyond IgE and related with the drug interaction with MRGPRX2 or IgG receptors, or mediated by their effect on some enzymes. However, the lack of a clear biomarker for characterizing them,

together with the difficulty of predicting cross-reactivity, makes the management of non-allergic reactions very complex.
Desensitization is standard intervention in allergic patients who need the drug. It is successful in IgE-mediated DHRs but its value on non-IgE-mediated DHRs is not well-known.
Summary Further research is needed to identify the mechanism involved in DHRs considering that IgE and non-allergic reactions cannot be mutually exclusive and can happen simultaneously, increasing their severity. It is crucial the identification of biomarkers in non-allergic reactions.

Introduction

Drug hypersensitivity reactions (DHRs) include those adverse reactions induced by a drug that involves the stimulation of the immune system [1••]. DHRs classification is a matter of debate. They can be classified according to the time interval between drug administration and appearance of the symptoms as immediate (less than 1 to 6 h), mainly induced by an IgE- or IgG and complement-mediated mechanism, and non-immediate (more than 1 h), often associated with a delayed IgG-mediated or T cell–dependent mechanism. There is an overlap in those reactions occurring between 1 and 6 h classically named accelerated reactions [2, 3••]. Although the mechanism involved in these reactions is not completely understood, it depends on the culprit drug and is often mediated by T cell [3••]. According to the mechanism involved, these reactions can also be classified in (i) *allergic reactions*, immunologically mediated [4]; (ii) *p-I reactions*, or pharmacological stimulation of immune receptors, in which the hapten directly binds to HLA or TCR proteins; and (iii) *pseudo-allergic or non-allergic reactions*, in which a variety of clinical entities with different mechanisms are included [1••].

Although both drug allergic and pseudo-allergic or non-allergic reactions are named immediate reactions and can induce similar clinical manifestations, the underlying mechanism is different [1••]. It is therefore important to differentiate between them since the management and the alternative indications will be different.

There are some aspects that may differentiate between allergic reactions from non-allergic reactions. The time onset of the reaction is similar; however, IgE-mediated reactions are usually faster, occurring in the first 15 min after drug intake, while non-allergic reactions occur within minutes or hours. Another key point to distinguish between both mechanisms is the necessity of prior sensitization, essential to develop an IgE-mediated reaction, whereas non-allergic reactions can be elicited upon first contact with the drug. Both types of reactions also differ in the usefulness of the diagnostic methods since the in vivo and in vitro test commonly used in the diagnosis of IgE-mediated reactions, such as skin tests (STs), immunoassays, or basophil activation test (BAT), are of low value for diagnosing non-allergic reactions. Finally, there are also differences in the management of both types of reactions and the search for alternative drugs. In IgE-mediated reactions, cross-reactivity is related to structural similarities and usually occurs with drugs of the same pharmacological group. However, in non-allergic reactions, cross-reactivity spectrum is very broad and not related to the chemical structure [1••].

In this review, we are going to describe the current knowledge about both IgE- and non IgE-mediated immediate DHRs, including drugs involved, related mechanisms, and possible biomarkers.

IgE-mediated DHRs

Drugs, as low molecular weight compounds, follow the hapten model since they need to bind to proteins for interacting with the immunological system [5].

This concept was proposed by Landsteiner and Jacobs [6] and is considered to be the mechanism by which most drugs interact with the immune system, leading to allergic reactions [7]. According to this hypothesis, the adduct formed by the binding of the drug or drug metabolites to a carrier protein (the antigenic determinant) would be responsible for inducing the allergic reaction through the production of specific IgE (sIgE) antibodies or T cells [8•, 9]. This reaction requires a prior exposure to the drug to induce the production of specific antibodies or T cells.

IgE-mediated reactions usually appear within the first hour after drug intake and can manifest themselves as urticaria, angioedema, bronchospasm, anaphylaxis, and anaphylactic shock when cardiovascular collapse appears [3••]. They begin with a sensitization phase, in which dendritic cells process the adducts and present them to lymphocytes with a Th2 phenotype. Those Th2 lymphocytes induce plasma cells to produce drug-sIgE which can bind to specific receptors, FcεRI, on the surface of basophils and mast cells. In subsequent contacts with the adduct, the simultaneous recognition of the drug by at least two adjacent sIgE, initiates a complex intracellular signaling cascade that leads to degranulation and the release of preformed mediators such as histamine, tryptase, and cytokines. These mediators are responsible for the allergic symptoms and promote the activation of other inflammatory cells and the production of other mediators such as prostaglandin D2 (PGD2) and cysteinyl leukotrienes, related to the amplification of the allergic reactions [4]).

Currently, two main mechanisms of basophil degranulation have been proposed. One of them implies the formation of small vesicles from the histamine-containing granules, which are rapidly shuttled to the plasma membrane [10, 11], and are related to the upregulation of CD203c molecules on the cell surface [12]. This mechanism, called piecemeal degranulation, is fast and would be linked to the development of the most severe reaction, which is anaphylactic shock [13••, 14]. The second mechanism, called anaphylactic degranulation, is slower than piecemeal degranulation and would be mediated by the fusion of the main histamine-containing granules and the plasma membrane, releasing the entire contents to the extracellular space and exposing CD63 on the surface of basophils [12]. This mechanism would be related to the development of anaphylaxis [15]. Recent data have shown that the basophil degranulation mechanisms could be different depending on the drug involved in the reactions, being more related to CD203c in patients with hypersensitivity to moxifloxacin, and to CD63 in hypersensitivity induced by other quinolone as ciprofloxacin or omeprazol [13••, 16].

The drugs mainly involved in IgE-mediated DHRs are antibiotics (as betalactams and quinolones) and neuromuscular blocking agents (NMBAs), although many other drugs, including chemotherapeutic agents, and some non-steroidal anti-inflamatory drugs (NSAIDs), can also elicit this type of reactions [17–21].

Diagnosis of IgE-mediated DHRs

The best biomarker for diagnosing these reactions is the drug sIgE, which can be determined by both in vivo and in vitro tests. The in vivo allergological work-up includes the performance of a detailed clinical history, often difficult to obtain in drug allergic patients [22], STs, and

drug provocation testing [23]. STs are considered as the most validated in vivo method for diagnosing immediate reactions [24]. However, they are not standardized for all drugs [18, 25] and in some cases have low sensitivity [26••] or show equivocal results as it happens with fluoroquinolones [27]. Due to these limitations, drug provocation tests are the "gold standard" in drug allergy diagnosis. However, this procedure is time-consuming and not free of risk for the patient, especially in those with severe symptoms [28]. Therefore, in vitro tests, although less sensitive, are the only alternative to in vivo testing in some cases [24]. The most used in vitro test is immunoassay, based on the quantification of drug sIgE in the patient serum using both commercial methods, such as ImmunoCAP FEIA, and in-house techniques, such as radioallergosorbent test (RAST) [29]. However, they are only available for a limited number of betalactams, NMBAs and chlorhexidine [26••] and their sensitivity is not optimal for most drugs, ranging from 36 to 77.7% [14, 15, 30–32]. BAT is an emerging technique for the diagnosis of DHRs and is based on the determination of basophil activation after drug stimulation using flow cytometry techniques [26••]. BAT is a functional test that provides the opportunity to evaluate DHRs induced by a great variety of drugs for which no other in vitro test is available, like clavulanic acid (CLV), omeprazol, and chemotherapeutics [16, 33••, 34, 35, 36••, 37]. Nevertheless, BAT can improve the diagnostic sensitivity since it has shown to be complementary to in vivo testing and even to other in vitro tests [26••]. Finally, although less investigated, some studies used histamine release test (HRT), based on the detection of histamine release by human basophils after incubation of blood with the drug. Recently, HRT has been used for the evaluation of selective reactions to CLV [38], showing a sensitivity of 55% and a specificity of 85%.

Since drugs can also interact with mast cells and basophils inducing non-IgE-mediated activation/degranulation [39], it may be necessary to confirm the activation pathway when immediate reactions are evaluated. One indirect way to confirm that the DHR is IgE-mediated, is to measure the rate of decrease of positive results in sequential immunoassays and BAT, as the clearance of sIgE overtime can affect the results of the tests [15, 20, 33••, 40]. Another method, specific for BAT, is to preincubate the cells with an inhibitor of the IgE signaling pathway such as wortmannin [15, 16, 21, 34, 41], which results in a reduction of basophil activation only when IgE is involved.

Management of IgE-mediated DHRs

A precise diagnostic of DHRs should help not only to identify the culprit drug that must be avoided but also to find safe alternatives for future treatments. Finding treatment options is especially important for patients with hypersensitivity to antibiotics who have a dramatic reduction of the therapeutic possibilities for managing infectious diseases and high risk of bacterial resistance induction.

Cross-reactivity, in IgE-mediated DHRs, is related with the similarity of the chemical structure and produced at very low doses due to the

high affinity of the drug-sIgE [1••]. Cross-reactivity has been widely reported for the most important drugs involved in these reactions, such as BLs and NMBA [42–49]. As alternatives, clinicians can prescribe drugs with lower similarities in their structures, e.g., for betalactams, the prescription of third/four generation of cephalosporins is safe in penicillin allergic patients [42].

Non-allergic reactions

There are some aspects in DHRs that may suggest that a non-IgE-mediated mechanism is involved, such as the development of the reaction after the first exposure, the low level of drug sIgE, and the low in vitro basophil activation even in severe reactions. These reactions, denominated non-allergic reactions, although frequently clinically indistinguishable from the IgE-mediated ones, can be mediated by several mechanisms. These reactions are thought to be produced by the interaction of the drug with inflammatory cells as mast cells, basophil, and neutrophils through different mechanisms: through the Mas-related G protein-coupled receptor member X2 (MRGPRX2) in the case of fluorquinolones and NMBAs; through IgG receptors in the case of biological agents; or interacting with enzymes as cyclooxygenase (COX) in NSAIDs [50, 51••] (Fig. 1).

Reactions mediated by MRGPRX2

Recently, a new receptor in mast cell surface that may be involved in immediate reactions to some drugs, has been identified by McNeil et al. [51••, 52]. Mast cells can be activated in an antibody-independent manner through the MRGPRX2 by a range of cationic substances called basic secretagogues, which include inflammatory peptides and polycationic molecules, such as 48/80. All these molecules that interact with MRGPRX2 contain a tetrahydroisoquinoline (THIQ) motif. Interestingly, NMBAs and fluoroquinolones, some of the drugs frequently involved in hypersensitivity reactions, have this motif and are able to activate mast cells after the interaction with this receptor in wild type mice but not in *MrgprX2* knock-out mice [51••, 52, 53]. The potential involvement of this receptor in DHRs was proved again by the results of experiments performed in human mast cell lines (LAD-2), where the knockdown of *MRGPRX2* gene significantly reduced mast cell activation evoked by basic secretagogues and drugs associated with pseudo-allergic reactions [54••] but not with IgE stimulation [51••].

The interaction of the drug with MRGPRX2 induces the release of histamine, β-hexosaminidase, tumor necrosis factor (TNFα), PGD2, and other inflammatory mediators, that all together are able to reproduce the same symptoms observed in allergic reaction. However, the degranulation process of the inflammatory cells differs from those induced by IgE-mediated mechanisms. Activation of the MRGPRX2 receptor induces a quick secretion of small granules, while FcεRI-dependent degranulation results in a more gradual degranulation, with longer and heterogeneous granules. These differences in the degranulation process are mirrored in vivo, with a faster and more localized reaction induced by MRGPRX2 and a more intense, prolonged and systemic reaction triggered by the FcεRI receptor [39].

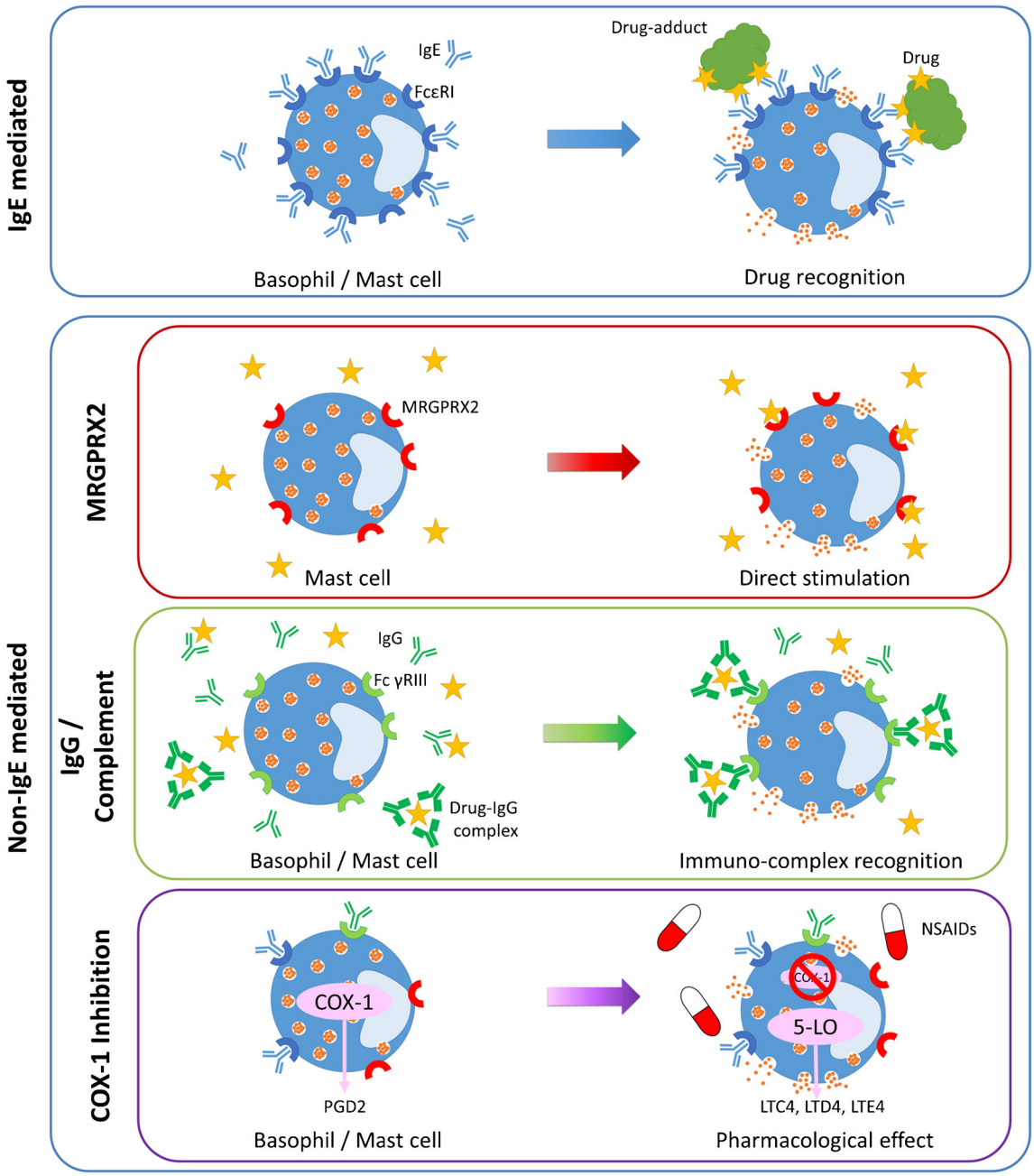

Fig. 1. Mechanisms involved in IgE- and non-IgE-mediated DHR. In IgE-mediated DHR, the adduct formed by the drug and a carrier protein is recognized by the sIgE on the surface of basophils and mast cells inducing its degranulation. In non-IgE-mediated reactions, several mechanisms can be involved: (i) the recognition of the drug by the mast cell receptor MRGPRX2; (ii) the formation of IgG-drug immunocomplex; (iii) the pharmacological effect of inhibitors of COX-1 pathway.

Besides quinolones and NMBAs, other drugs such as opioids, vancomycin, radio contrast media, and dextrans have chemical characteristics similar to the biological ligand of MRGPRX2, the presence of a THIQ motif, and have shown

to be able to activate mast cells via this receptor [52, 54••, 55]. The existence of this mechanism may explain the high rate of anaphylactic reactions upon first exposure as well as the high rate of cross-sensitization, mainly demonstrated by positive STs results to various NMBA despite their different chemical structures [56••]. However, beside this off-target occupation of the MRGPRX2 receptor by these drugs, some other factors in predisposed individuals that could have a cumulative effect, must be involved to be clinically relevant [56••].

The diagnosis of these reactions can include a similar allergological work-up as for immediate reactions. However, the results of STs for these reactions are not so valuable and must be interpreted with caution since a high rate of false positive results has been described for quinolones, maybe due to the local activation of mast cells via MRGPRX2 [27]. Moreover, for NMBA a high rate of false negative results has been described [54••]. Recently, a cell line of rat basophilic leukemia (RBL) cells that stably express the human MRGPRX2 receptor has been developed [57], establishing a potential model for future screening and evaluation of new drugs as potential inductor of allergic reactions [58]. However, as no clear biomarkers have been yet identified, there is no in vitro test to diagnose these types of reactions. Moreover, although basophils are thought to be the equivalent of mast cell in peripheral blood, it has been shown that basophils constitutively express intracellular MRGPRX2, but barely express this receptor on their surface [59]. Thus, it is unlikely that BAT will be of value for evaluating reactions occurring from off-target occupation of this receptor.

Reactions mediated by IgG or complement

The existence of anaphylactic reactions mediated by IgG has been demonstrated in mouse models [60]. In these types of reactions, the drug is recognized by specific IgG bound to FcγRIII on the surface of basophils, macrophages, or neutrophils. This interaction leads to the release of platelet-activating factor (PAF), among other mediators, driving anaphylaxis [61, 62]. The presence of IgG immunocomplexes can also activate the complement pathway, leading to the release of C3a, C5a, and C5b-9. Those fractions can induce the activation of mast cells, basophils, and other cells via their specific receptors, causing degranulation and mediator release [62]. In humans, this mechanism has not been fully established, although several evidences point to its existence. Drugs solubilized in therapeutic liposomes or lipid-based excipients can activate complement pathway under physiological conditions [62]. Muñoz-Cano et al. suggest that both IgG and neutrophils may be involved in human anaphylaxis. They observed in a group of patients with food anaphylaxis induced by lipid transfer proteins (LTP) an increase of specific anti-LTP IgG1 and IgG3 was observed [63••, 64].

The assessment of the levels of PAF could be an indicative of the development of these non-IgE-mediated reactions. In fact, some studies have correlated the severity of anaphylaxis to the levels of PAF and of PAF acetylhydrolase (PAF-AH), in charge of PAF inactivation [65]. In this way, patients with higher PAF levels and lower PAF-AH activity had 27 times more risk of severe or fatal anaphylaxis than patients with normal levels [66, 67].

Moreover, some studies have shown that PAF is an essential mediator in anaphylaxis [66, 67], especially in those induced by biological agents, where

there is no detectable sIgE but high levels of sIgG have been found. This observation has been made in patients transfused with IgA [68, 69], or treated with infliximab or adalimumab [70–72] and other monoclonal antibodies [73–75], where high quantities of the drug are administered. However, there must exist some individual predisposition to suffer this type of reaction as an increased frequency of a gain-of-function allele of the stimulatory FcgRIIA has been demonstrated in these patients [76]. Indeed, in infliximab treated patients, the presence of high levels of specific IgG has been related to an increased risk of anaphylaxis [77].

Reactions mediated by COX inhibition

Non-IgE-mediated hypersensitivity reactions to NSAIDs or cross-reactive hypersensitivity are mainly induced by its pharmacological effect. Although considerable clinical information is available for these NSAIDs induced hypersensitivity reactions [78–83], the pathogenic mechanism remains unclear.

These drugs are inhibitors of the cyclooxygenase-1 (COX-1) activity, an enzyme involved in the arachidonic acid (AA) pathway [84]. The inhibition of this enzyme shunts, in susceptible individuals, the AA metabolism from prostaglandins (PGs) towards the 5-lipoxygenase pathway, leading to the overproduction of cysteinyl-leukotrienes (CysLTs; LTC4, LTD4, and LTE4) and the reduction of the synthesis of PGs and tromboxanes [85, 86]. These reactions may affect skin and/or respiratory airways, although skin is the most common organ affected [82].

This type of reactions, classically called cross-intolerant reactions, normally involves several drugs chemically unrelated, mainly with high COX-1 inhibitory activity such as acetylsalicylic acid, ibuprofen, diclofenac, and naproxen [50]. The diagnosis of cross-intolerant reactions is complex and mainly based in a well-documented clinical history and the performance of oral provocation tests to confirm or rule out tolerance to different drugs. As these reactions are not mediated by IgE or T cells, STs are of no value, as well as in vitro tests such as sIgE determination or BAT [87].

Management of non-allergic reactions

As the same drug can elicit both IgE and non-IgE-mediated reactions, determining the underlying mechanism is extremely relevant. One of the main characteristics of non-allergic reactions is that they are dose-dependent and elicited by higher doses of drug compared to IgE-mediated reactions. Moreover, as non-allergic reactions can be induced by functional off-target receptor(s) on inflammatory cells, they are not predictable and can be produced at the first contact with the drug, not requiring a prior sensitization.

The recommendations for alternative drugs in pseudo- or non-allergic reactions are more complex than the IgE-mediated ones, since they are produced by groups of drugs that do not share structural similarities.

Treatment of IgE and non-IgE-mediated DHRs: desensitization

Desensitization is currently considered a standard intervention in drug allergic patients who need the drug they are allergic to as first-line therapy [88]. This procedure permits the safe reintroduction of critical antibiotics and other drugs

in patients who had developed DHRs mediated by mast cell activation, whether the mechanisms are IgE or non-IgE-mediated [89••]. Although the efficacy and safety of desensitization has initially been established for IgE-mediated DHRs [88], there are several reports of successful desensitizations in patients with immediate reactions to taxanes and other chemotherapies in which IgE mechanism cannot be demonstrated [89••]. Besides, there are reports about successful desensitizations to NSAIDs in aspirin-exacerbated respiratory disease patients, resulting in an improvement of the sense of smell, reduction of nasal polyps regrowth, and a better control of asthma symptoms [88, 90, 91].

Desensitization procedure consists in the administration of incremental escalated sub-optimal doses of the culprit drug until the required dose is reached, inducing a temporary tolerance to the drug [89••]. Desensitization involves the induction of inhibitory mechanisms at low drug doses, such as impairments of receptor internalization, calcium flux, degranulation, early de novo synthesis of lipid mediators, and late cytokine production, as well as inhibition of the IgE signal transduction [89••]. Whether these mechanisms also drive desensitization in non-IgE-mediated DHRs needs further research.

Nevertheless, knowing the underlying molecular mechanisms, comorbidities, STs results, and potential genetic markers would improve risk stratification, precisely identifying the most suitable individuals for desensitization and minimizing the risk of a new reaction during the procedure [89••].

Conclusions

DHRs occurring 1 to 6 h after drug intake, immediate reactions, can be produced by different mechanisms. Its elucidation is essential, not only for the accuracy on the diagnosis, but also for establishing recommendations for future treatments and identifying safe drug alternatives.

IgE-mediated reactions are faster and cross-reactivity is produced by drugs with similar chemical structures. Diagnostic methods using the determination of sIgE, as the main biomarker, have proved to be useful, although with different degree of sensitivity depending on the drug involved in the reaction.

Non-IgE-mediated reactions can happen several hours after the drug administration and the identification of alternative non-cross-reacting drugs is more complex. The current diagnostic methods, in vivo (STs) and in vitro (immunoassays and BAT), are not useful. Thus, there is an urgent need for searching for new biomarkers that may identify these types of reactions.

Nowadays, the only treatment for DHRs, either IgE or non-IgE-mediated, is desensitization, which has demonstrated its usefulness, especially for chemotherapeutics, antibiotics, and NSAIDs.

Further research is needed to identify the mechanism involved in DHRs considering that IgE and non-IgE reactions can not be mutually exclusive and, moreover, simultaneous occurrence of IgG- and IgE-mediated anaphylaxis or synergistic effect of direct mast cell activation by drugs with antibody-dependent activation could occur and increase the DHRs severity. In summary, all this knowledge is extremely relevant and would lead to the development of

therapeutic strategies aimed to block specific activation pathways that may prevent, or at least decrease the severity of DHRs.

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Compliance with Ethical Standards

Conflict of Interest
Cristobalina Mayorga, R. Muñoz-Cano, A. Rodríguez-Nogales, R. Fernandez-Santamaría, and T. D. Fernandez declare that they have no conflicts of interest.

Human and Animal rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

Publisher's Note

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
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