Chapter 3

Principles of Vaccination

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Abstract

While many of the currently available vaccines have been developed empirically, with limited understanding on how they activate the immune system and elicit protective immunity, the recent progress in basic sciences like immunology, microbiology, genetics, and molecular biology has fostered our understanding on the interaction of microorganisms with the human immune system. In consequence, modern vaccine development strongly builds on the precise knowledge of the biology of microbial pathogens, their interaction with the human immune system, as well as their capacity to counteract and evade innate and adaptive immune mechanisms. Strategies engaged by pathogens strongly determine how a vaccine should be formulated to evoke potent and efficient protective immune responses. The improved knowledge of immune response mechanisms has facilitated the development of new vaccines with the capacity to defend against challenging pathogens and can help to protect individuals particular at risk like immunocompromised and elderly populations. Modern vaccine development technologies include the production of highly purified antigens that provide a lower reactogenicity and higher safety profile than the traditional empirically developed vaccines. Attempts to improve vaccine antigen purity, however, may result in impaired vaccine immunogenicity. Some of such disadvantages related to highly purified and/or genetically engineered vaccines yet can be overcome by innovative technologies, such as live vector vaccines, and DNA or RNA vaccines. Moreover, recent years have witnessed the development of novel adjuvant formulations that specifically focus on the augmentation and/or control of the interplay between innate and adaptive immune systems as well as the function of antigen-presenting cells. Finally, vaccine design has become more tailored, and in turn has opened up the potential of extending its application to hitherto not accessible complex microbial pathogens plus providing new immunotherapies to tackle diseases such as cancer, Alzheimer's disease, and autoimmune disease. This chapter gives an overview of the key considerations and processes involved in vaccine development. It also describes the basic principles of normal immune respoinses and its their function in defense of infectious agents by vaccination.

Key words Vaccine, Vaccination, Immunology, Pathogen, T cell, B cell, Infectious disease

1 Introduction

Vaccination is one of the most effective medical interventions to reduce morbidity and mortality of infectious diseases. The main principle of vaccination is the proactive induction of a protective immune response by mimicking the natural interaction of an infectious pathogen (bacteria, viruses, etc.) with the human immune system (Fig. 1).

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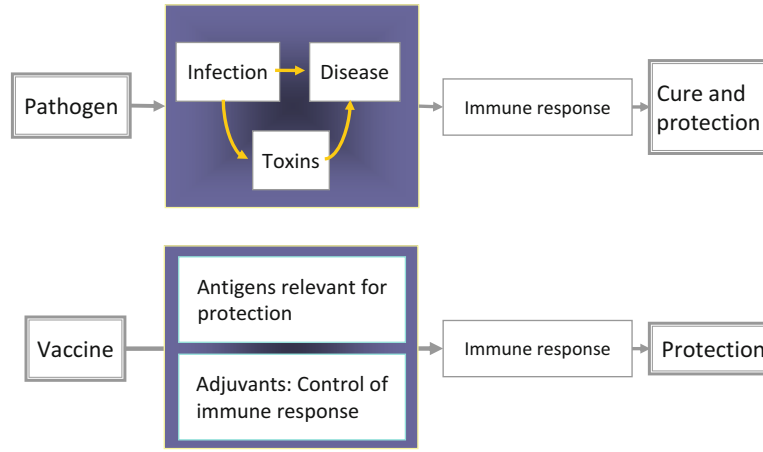

Fig. 1 Principles of vaccine development. Adapted from Moser, Leo: Key concepts in immunology. Vaccine 28S (2010) C2–C13

Table 1
Principles of vaccine design

Etiology of infectious diseases
• Biology and epidemiology of infectious agents
• Replication, polymorphism, immune evasion
• Microbial virulence factors
• Microbial sanctuary
Pathogenesis of the infectious disease
• Mode of infection
• Toxin-mediated symptoms
• Quality of naturally occurring immune response
• Capacity to evade host immune responses
Identification of protective immune responses
• Definition of relevant antigenic structures
• Evaluation of antigen processing by antigen-presenting cells
• Evaluation of (protective) B and/or T cell responses

In contrast to natural infection vaccines ideally achieve their protective effects without clinical symptoms of disease or side effects.

Vaccine design in principle builds on the structure and biological properties of an infectious agent. It is of utmost importance for vaccine developers to understand the etiology, epidemiology, pathogenesis, and immunobiology of the target infection [1] (Table 1). Moreover, an ideal vaccine should also have the capacity

to induce immune responses that provide cross-protection against variant strains of the infectious microorganism. To do so, the vaccine must elicit all the steps leading to immune activation by promoting an adequate effector mechanism, involving mediators and cellular responses, which are tailored to the specific disease.

At all times in varying degrees the development of vaccines was based on close observation of natural phenomena [2]. Early vaccinologists including Edward Jenner (*see* below) deducted their concepts from the observation that under certain conditions individuals were spared from highly contagious diseases. Over the last two centuries vaccination was strongly endorsed by the progress in biological sciences, the emergence of biochemical techniques, and the discoveries in immunology, genetics, and molecular biology. The techniques available in the late twentieth century further facilitated the development of new vaccine concepts such as subunit vaccines (purified protein or polysaccharide), DNA or mRNA vaccines, or genetically engineered antigen components based on reverse vaccinology [3]. However, the advantages of modern vaccine concepts are often associated with specific drawbacks, including the fact that highly purified vaccine antigens often provide only weak immunogens. Moreover, there are challenging diseases such as malaria, tuberculosis, or HIV/AIDS that still remain out of reach of classical vaccine design. To overcome these impediments, lately new approaches based on innovative adjuvant formulations have been established [4]. Following the recognition of the important role of the innate immunity for the induction of an adaptive immune response new adjuvants were developed that have the ability to modulate the immune response, increasing the level of immune activity to that typically seen with original live attenuated or killed vaccines. Thus, modern vaccines may have the potential to compensate even for limitations of naturally occurring immune responses.

2 A Brief History of Vaccination

Already in the ancient world it was common knowledge that an individual rarely was infested twice with the same disease. This observation led to the practice of inoculation that has been documented in China more than 1000 years before Jenner's remarkable studies [2]. Even the term "immunity" was used in reference to plague during the fourteenth century. Progress in natural sciences and the development of experimental techniques during the eighteenth century led to the systematic use of inoculation to fight smallpox, one of the most serious threats during that time. In the early eighteenth century variolation, the transmission of small, presumably sublethal volumes of liquid from smallpox pustules was introduced to England by Lady Mary Wortley Montagu. Lady Montagu survived infection with smallpox herself. Impressed

with the method of variolation she ordered the embassy surgeon, Charles Maitland, to inoculate her 5-year-old son. After her later return to London in 1721, Lady Montagu introduced the method to the physicians of the royal court. Thereafter variolation became quickly popular among physicians in Europe. However, variolation was not without risks. In average 2–3 % of variolated persons died from the disease but the mortality associated with variolation was ten times lower than that associated with naturally occurring smallpox.

Modern concepts of vaccination date back to 1796 when Edward Jenner based on empirical observation used liquid from pustules of cowpox to induce protective immunity in human individuals. Today the use of cowpox as a vaccine is considered to be the landmark of modern vaccination concepts. Edward Jenner recognized that milkmaids infected by cowpox, a generally harmless infection for humans, were rendered immune to smallpox. In 1796 Jenner deliberately inoculated people with small doses of cowpox (vaccinia) from pustules and successfully demonstrated that protection against smallpox could be achieved. Jenner termed this preventive measure "vaccination" and over the following decades inoculation against smallpox using cowpox became widely accepted in Europe. While Jenner at his time neither understood nor could explain the biological basis of "vaccination," his concept was successful and provided protection from smallpox apparently due to cross-immunity between cowpox and smallpox.

Until the end of the nineteenth century, diseases were believed to be caused by invisible microbes which were "spontaneously generated" in response to "bad air" and other environmental triggers, as well as a belief that imbalance in the body caused what were actually infectious illnesses. Progress in microbiology and virology since the late nineteenth century elucidated the modern concept of communicable diseases. Pasteur and Koch established that microorganisms were the true cause of infectious diseases. These discoveries led to the science of immunology. Hence further advances in vaccinology were gained from an increasing understanding of the etiology of infectious diseases and host-pathogen interactions. Pasteur challenged the spontaneous generation theory of microbes while Koch demonstrated that infectious agents transmit diseases. Koch defined four postulates which established an individual agent as the cause of a disease. In addition, in the late 1870s Pasteur developed the first attenuation procedure for pathogens. Pasteur's approach provided microorganisms less pathogenic but still immunogenic. Using animals as a live propagating medium, Pasteur and his team were able to produce attenuated rabies viruses of different strengths of which the weakest could be used to prepare a vaccine. In 1885, the first human individual was vaccinated with a live, attenuated rabies vaccine. However, due to technical

limitations of vaccine production at that time, fatal cases of rabies in vaccinated individuals occurred.

At the end of the nineteenth century, many of the fundamental aspects of vaccinology were established due to the pioneering work of Pasteur and Koch. Probably the most important advance was the insight that the administration of pathogens, either attenuated or killed, resulted in protection against the disease caused by the respective non-treated pathogen. The first inactivated vaccines, developed in the 1890s, were directed against the typhoid and cholera bacilli [5]. Other vaccines consisting of killed whole pathogens, produced in the early twentieth century, were directed against pertussis [6], influenza [7], and typhus. These were followed by inactivated vaccines directed against polio (IPV) [8], rabies, Japanese encephalitis, tick-borne encephalitis [9], and hepatitis A [10].

Although inactivated vaccines exhibit a lower risk of vaccine-associated disease than live vaccines, their efficacy can be reduced by the same factors, i.e., circulating antibodies (maternal antibodies) or concomitant infection. Moreover, multiple doses of inactivated vaccines are generally needed to provide sufficient stimulation of the immune system to induce durable immune responses. This observation led to the introduction of aluminum compounds as vaccine adjuvants (from the Latin word adiuvare, meaning "to help or aid"). Still today aluminum salts represent the most frequently used adjuvant system (*see* below). Further progress in biochemistry facilitated the development of inactivated vaccines based on purified toxins. The first subcellular vaccines made available in the 1920s used diphtheria and tetanus toxoids [2]. As technology improved, it became possible to purify protein or polysaccharide subunits from infectious organisms to develop increasingly specific vaccines.

Another important milestone was the development of sophisticated ways to culture and propagate infectious pathogens, like viruses, ex vivo. Based on these new techniques the development and production of purified attenuated viral pathogens as live vaccines became possible. Typical examples of vaccines that use passage in artificial media or cell culture as means of attenuation include the oral polio virus (OPV) [11] or measles, mumps, rubella, and varicella vaccines [12] as well as the Bacille Calmette-Guérin (BCG) tuberculosis vaccine [13].

Recent years have been characterized by impressive progress in the fields of immunology and molecular biology as well as important technical improvements concerning fermentation and purification. Building on the improved knowledge of the principles of host-pathogen interactions, the host's immune response today can be dissected in order to identify the individual antigenic structures that are most relevant to initiate protective immunity. The appropriate antigens are isolated as subcomponents of pathogens and subsequently produced in large quantities either by purification or

by in vitro construction using molecular genetic technologies. Moreover, innovative adjuvants have been introduced that specifically modify and augment those aspects of the immune response that are most appropriate for protection. These adjuvants also have the potential to generate long-lasting immunological memory to maintain protection.

During the last 100 years vaccine development has evolved from an empirical approach to one of more rational vaccine design where careful selection of antigens and adjuvants is key to the desired efficacy for challenging pathogens and/or challenging populations. Modern vaccine design needs to consider factors beyond target antigen selection to improve immunogenicity while conserving a favorable reactogenicity and safety profile [1]. With new vaccine technologies currently emerging, it will be possible to custom-design many vaccines for optimal efficacy, low reactogenicity, and excellent safety profiles in the near future.

3 Basic Concepts of Vaccine Immunology

The primary goal of vaccination is the induction of protective immunity against disease-causing infectious pathogens, i.e., microorganisms like bacteria, viruses, or fungi. To achieve this objective vaccines mostly are designed to address natural defense mechanisms and activate the immune system in a manner similar to natural infections. Vaccine development, therefore, strongly depends on our understanding of the human immune system [14].

The human immune system comprises two major compartments: the innate and the adaptive immune system (Fig. 2). Innate and adaptive immunity work sequentially to identify invading

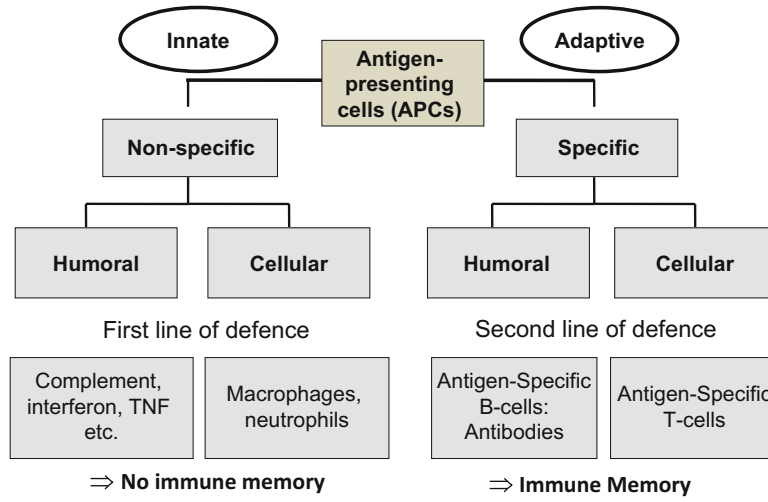

Fig. 2 Innate and adaptive immunity—overview

pathogens and initiate the most effective defense response. The interaction of innate and adaptive immunity is crucial to generate and maintain a protective immune response. Especially specialized antigen-presenting cells (APCs) are important to bridge the two compartments of the immune system [15].

4 Innate Immunity

The innate immune system represents a first line of host defense against pathogens that surmount the body's physical and chemical barriers (e.g., skin, ciliated epithelia, mucous membranes, stomach acids, and destructive enzymes in secretions). Innate defense mechanisms are mediated by cellular effector cells and noncellular effector molecules such as complement or lysozyme. Cellular elements of the innate immune system are generated in the bone marrow and migrate into blood and different tissues of the body. Tissue-residing (e.g., macrophages and dendritic cells) and "mobile" phagocytic cells (e.g., neutrophils, eosinophils, and monocytes) as well as natural killer cells represent major cellular elements of the innate immunity [16].

After invasion of a pathogen the innate immune system is responsible to detect, contain, and ideally eliminate the thread immediately. Innate immunity has only a limited number of receptor molecules available to fulfill this task. Pathogens are detected through molecular-sensing surveillance mechanisms via pattern recognition receptors (PRRs), expressed by cells of the innate immune system either on the cell surface or in intracellular compartments (i.e., DNA/RNA sensors). Typical examples of PRRs are the transmembrane Toll-like receptors (TLRs) which recognize pathogen-associated molecular patterns [PAMPs] that are shared by several pathogens (for example lipopolysaccharide expressed by all Gram-negative bacteria), thereby enabling the innate immune system to sense the occurrence of an infectious event [17]. For instance, TLR4 at the cell surface recognizes bacterial, whereas TLR9 is located intracellular and recognizes viral single-stranded RNA. PPRs sense danger signals and activate and augment proinflammatory gene expression in order to facilitate host defense capacity. Epithelial cells, fibroblasts, and vascular endothelial cells can also recognize PAMPs and activate innate immune cells when infected, stressed, or damaged. This is mediated by chemical messengers like cytokines and chemokines that are secreted by infected cells and/or innate immune cells to attract other resident and circulating innate cells to the site of infection.

Under some circumstances, pathogen elimination may be achieved by innate immune effectors alone without recruitment of a subsequent adaptive immune response. This can be accomplished by phagocytosis of pathogens and subsequent intracellular

destruction within intracellular vesicles containing oxygen radicals and digestive enzymes. Additionally, pathogens can be destroyed by soluble chemical factors secreted by innate immune cells or generated in the liver. Complement represents the most important and effective soluble effector system of innate immunity [18]. Complement proteins circulate in the blood in an inactive form. Comparable to the coagulation system the 25 complement proteins are activated in cascades. When activated, complement components fulfill several effector functions including the recruitment of phagocytes, the opsonization of pathogens to facilitate phagocytosis, and the removal of antibody-antigen complexes. The complement system also strongly promotes the effector function of the adaptive immune response by mediating lysis of antibody-coated pathogens. The innate immune response is enforced by chemotactic stimuli, released by infected epithelial and endothelial cells or other innate immune cells to recruit additional circulating cells from the bloodstream to the site of inflammation. While the defense provided by innate immune mechanisms in principle is sufficient to resolve an infection, during evolution many microorganisms have developed escape mechanisms to overcome the effectors of innate immunity. In most cases innate immunity will delay the invasion of pathogens, but intervention of the adaptive immune response is indispensable to overcome and finally clear an infection.

Although innate defense mechanisms are prearranged and fast reacting, they lack specificity and are not equipped to provide an immunological memory response. In consequence innate immunity alone is not sufficient for vaccine-related protective immune responses that depend strongly on the induction of immune memory responses [19]. Nevertheless, innate immunity fulfills an important role in the early detection of invading pathogens and subsequent activation of the adaptive immune response. The detection of pathogens and the phagocytosis of antigens by immature dendritic cells (DC) are important prerequisites to initiate adaptive immune responses. After ingestion of antigens immature DCs transform into antigen-presenting cells (APC) that migrate to the draining lymph node. The APC acts as a messenger to precisely define the nature of the perceived danger and convey this information to secondary lymphoid organs, where they activate the relevant adaptive immune response. Although vaccines in the end target the adaptive immune system, vaccine antigens must be recognizable by innate immune cells.

5 Adaptive Immunity

Adaptive immunity represents the second line of immunological defense. Antigen recognition by the adaptive immune system initiates a focused, highly specific immune response that results in

elimination of the pathogen and termination of the infectious disease. Moreover, in the course of an adaptive immune response antigen-specific memory cells are generated that will provide a faster and stronger immune response whenever the body is challenged by the same pathogen again in the future [19]. The cellular elements of the adaptive immune response are lymphocytes that are able to specifically recognize antigens, i.e., the components of an infectious pathogen "foreign" to the body and potentially dangerous. There are two main subsets of lymphocytes: B cells which initially develop in the bone marrow and T cells which are generated in the thymus. Activated B cells can produce and secret antigen-specific antibodies, i.e., proteins that will bind to antigens. T cells comprise of different types of lymphocytes that confer either regulatory or effector functions. T cells with regulatory function preferentially express the cluster of differentiation (CD) 4 cell-surface protein, and are referred to as CD4-positive T cells. Effector-T cells are characterized by the expression of the CD8 cell surface molecule.

In contrast to innate immune cells lymphocytes can express a huge diversity of antigen-specific receptor molecules (around several thousand billion) [20–22]. Antigen receptors are encoded by a set of genes that undergo multiple recombination events, eliciting the random generation of an extensive number of diverse receptor structures. The diversity of the receptor repertoire is further increased by individual changes and random gene insertions. The huge T and B cell repertoires of the human immune system provide the potential to recognize almost every naturally occurring antigenic structure. Initially the repertoire is maintained with single or very few cells expressing receptors that will recognize any given antigen, until individual clones are selectively expanded in response to a specific challenge. During the development of the adaptive immune system lymphocytes expressing receptors that potentially could recognize self-antigens are eliminated by a process named negative selection, while simultaneously cells that recognize non-self-antigens are positively selected.

6 T Cells

Each T cell expresses a unique antigen-specific receptor molecule (TCR). TCRs, however, cannot directly recognize complete pathogenic structures. Instead the TCR recognizes molecular fragments (small peptides derived from processing of larger protein antigens) that have to be presented in association with major histocompatibility complex (MHC) molecules at the cell surface of antigen-presenting cells (APC). In consequence, activation of T-lymphocytes strongly depends on the interaction with APCs. Professional APCs, derived from specialized phagocytes termed dendritic cells (DCs),

ingest pathogen-derived proteins. After phagocytosis the antigens are broken down and processed and the resulting peptide fragments are transported to the cell surface where they are embedded into MHC molecules. An individual T cell can only be activated by a peptide antigen for which it expresses the specific receptor. Moreover, besides its antigen specificity the TCR additionally can only interact with MHC molecules of its own tissue type. This quality is described as self-restriction and ensures that only cells of the same organisms will interact to mount an adaptive immune response.

T cells activated by antigen-bearing DCs express the CD4 cell surface protein and are restricted to recognize antigen in the context of MHC class II molecules. CD4+ T cells fulfill modulatory and effector functions by secreting soluble factors (cytokines) that exert direct antimicrobial properties or affect the activities of other immune cells. In most cases CD4+ cells will help other immune cells to perform their task and are, therefore, referred to as helper T cells (Th). Based on the types of cytokines the Th cells secrete and their abilities to assist other subsets of immune cells, several subpopulations of Th cells have been described. Th1 cells secrete mainly interferon-gamma (IFNγ), a cytokine known to limit pathogen survival. IFNγ also promotes the differentiation of cytotoxic lymphocytes (CD8+ cells *see* below) that are able to destroy cells infected by intracellular pathogens. T helper 2 cells produce various cytokines (interleukins [IL] IL-4, IL-5, IL-13) that preferentially activate innate immune cells (eosinophils, mast cells) especially facilitating the immune response to extracellular parasites (Fig. 3). Another subset, termed follicular T helper cell (Tfh) based on its tissue localization in follicular structures of lymph nodes, is characterized by the secretion of IL-21, a cytokine thought to favor the secretion of antibodies by antigen-specific B cells [23]. Finally regulatory T cells (Treg cells) belong to the CD4+ T cell subset. They inhibit immune or inflammatory responses by blocking the activity of effector T cells, helper T cells, and APCs. Treg are crucial to downregulate immune responses after an effective protective response, to maintain immunological self-tolerance process, and for the prevention of uncontrolled or chronic inflammatory responses.

T cells expressing the CD8 surface molecule represent T effector cells that have the capacity to eliminate cells infested with intracellular pathogens. Antigen recognition by CD8+ T cells depends on the fact that virtually all nucleated cells present fragments of intracellular proteins at their Surface-MHC-molecules as part fragments of intracellular proteins present externally derived antigen fragments in association with MHC class II molecules, non-immune cells use MHC class I molecules to present peptides derived from intracellular sources. Thus, cells infected by intracellular pathogens will express antigenic fragments of the pathogen in addition to the normal set of self-antigens. CD8+ T cells continuously screen MHC class I molecules to detect non-self-antigens indicative for an intracellular infection. Cells displaying high levels of

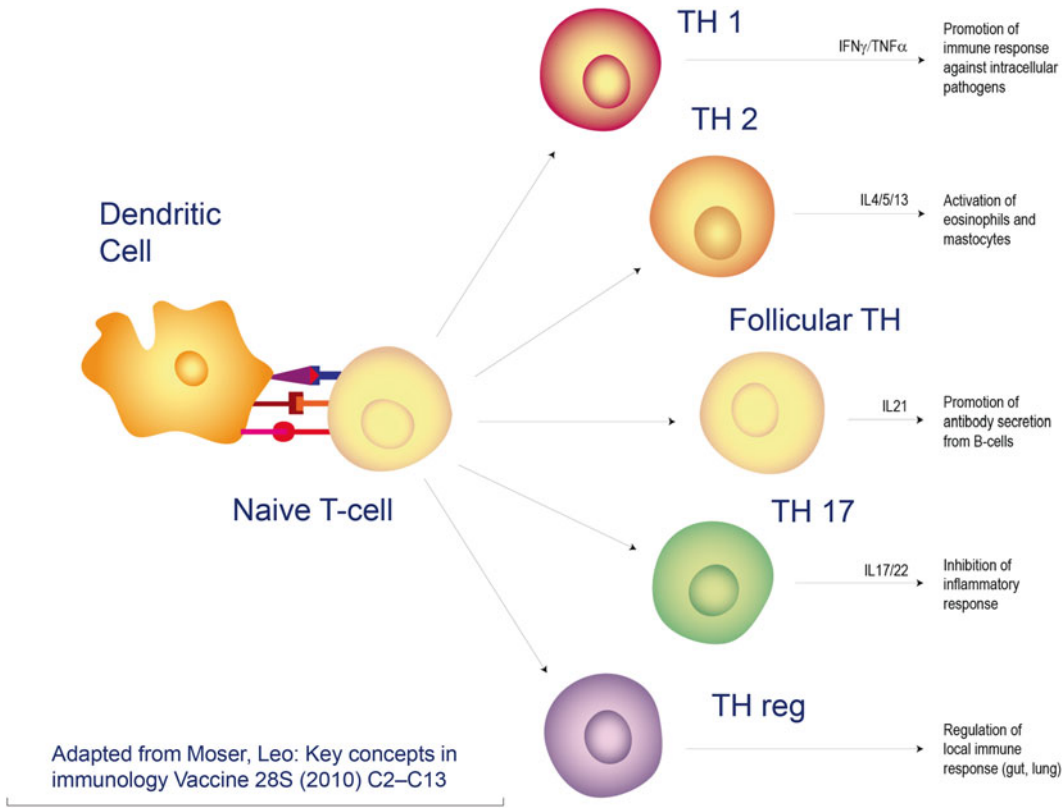

Fig. 3 Specialized T-helper cells

pathogen-derived peptides, e.g., in the case of a virus infection, subsequently will be killed by CD8+ T cells by secretion of cytotoxic factors. In addition, CD8+ T cells can inhibit viral replication without destroying the infected cells by producing cytokines that are able to interfere (interferon) with pathogen replication. CD8+ cytotoxic cells also can eliminate cells exhibiting abnormal host peptides, such as those presented by tumor cells, and therefore play an important role in the immune control of aberrant cell growth. Although CD8+ T cells can react directly to cells expressing non-self-antigen/MHC class I complexes, their optimal cytotoxic potential is achieved in the presence of cytokines produced by regulatory CD4+ T helper cells.

7 B Cells

B cells represent the second effector compartment of the adaptive immune response. Like T cells, each B cell expresses a unique antigen receptor (B cell receptor: BCR), which consists of a membrane-bound copy of the antibody molecule that can be secreted by the B cell after activation [24]. In contrast to T cell receptors the BCR

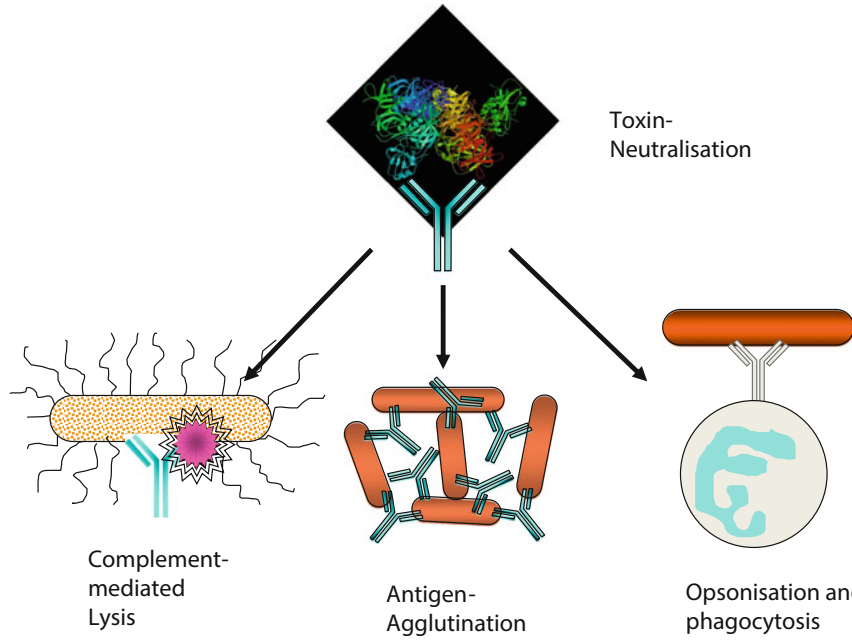

Fig. 4 Antibody-mediated protection

binds directly to molecular structures of pathogens with no need for previous antigen processing. Antigen binding by the appropriate BCR activates the B cell and induces proliferation and differentiation into plasma cells [25]. Plasma cells produce and secret large amounts of antibodies that are released in the blood and other body fluids. Antigen-specific antibodies are an important effector concept of additive immunity. Antibodies can facilitate phagocytosis or complement-mediated killing of pathogens or neutralize toxins by binding to their appropriate antigens (Fig. 4).

Antibody molecules consist of a "constant" fragment (Fc fragment), a structural feature common to all antibodies of a given isotype, and a "variable" region, which includes the region that defines the antigen specificity (Fab fragment). The constant part of the molecule exists in five different classes (isotypes) termed immunoglobulin [Ig] A, IgD, IgE, IgG, and IgM. The Ig isotype determines the ability of an antibody class to localize to particular body sites and to recruit the optimal effector cells. The variable region of the antibody exists in a huge number of randomly generated different molecular configurations. This BCR repertoire guarantees maximal capability to recognize diverse pathogenic antigen. Activation of B cells after the first encounter with an antigen and subsequent differentiation into plasma cells usually needs 10–14 days. Initially plasma cells will typically produce IgM-type antibodies. IgM antibodies are large molecules consisting of five bivalent antibody molecules linked together to exhibit ten binding regions. In the further course of the immune response antibody production

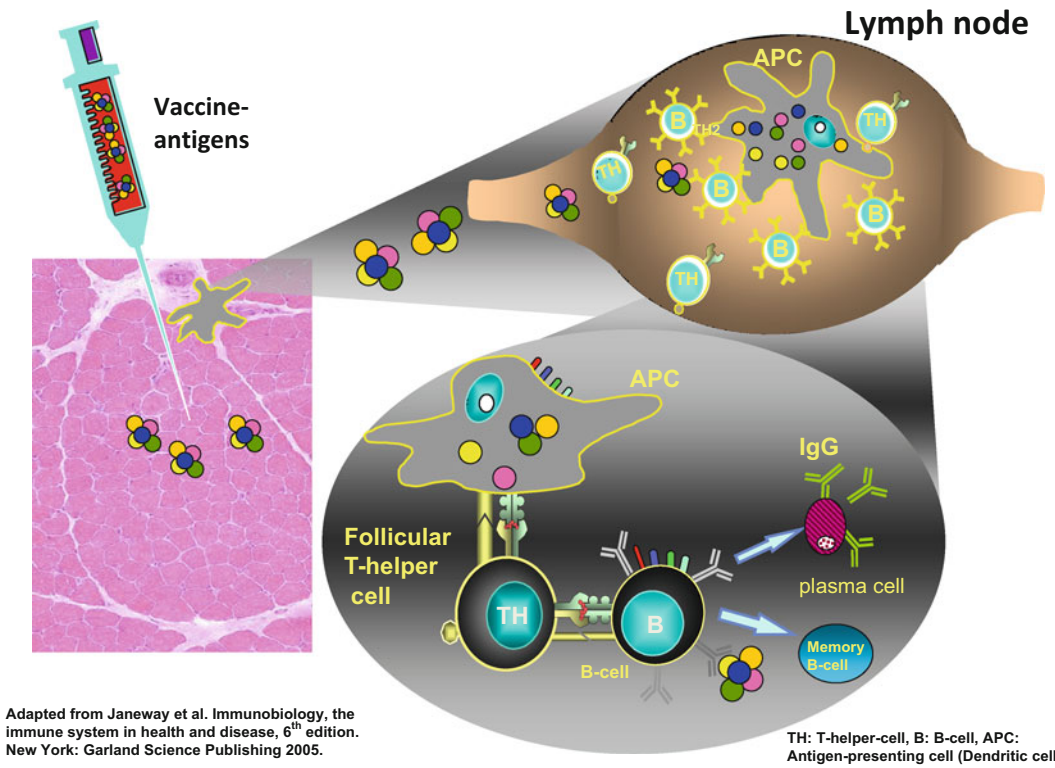

Fig. 5 Immune response after vaccination. Adapted from Janeway et al. Immunobiology, the immune system in health and disease, 6th edition. New York: Garland Science Publishing 2005

will switch to the IgG isotype, which also represents the major isotype of B cell memory responses [26]. Depending on the specific circumstances of B cell activation antibody production may switch to IgA which is secreted to mucus membranes or IgE, mainly for the defense of infections by parasites.

In most cases, optimal B cell activation and differentiation into antibody-secreting plasma cells will only be achieved when B and T cells are simultaneously activated by elements of the same pathogen (Fig. 5). T cell-independent direct activation of B cells occurs only in response to repetitive antigenic structures, such as carbohydrates found in bacterial walls. These T cell-independent immune responses are characterized by the secretion of low-affinity antibodies of the IgM type, lacking the typical memory response upon reexposure to the same antigen.

In these instances, activated B cells will recruit the help of T cells to mount an optimal response and to elicit immunological memory. After activation of the B cell by binding to a pathogen antigen the surface BCR-antigen complex will be internalized and elements of the antigen are processed and presented to an appropriate CD4+ T helper cell. The interacting CD4+ T cell will differentiate into a follicular T helper cell in order to provide helper signals

for the B cell. T cell-dependent B cell responses are characterized by the secretion of high-affinity antibodies and a large spectrum of isotypes (in particular IgG). The quality of antibody response has a bearing on protection, e.g., the antigen binding capability of antibodies (affinity, avidity) and the dynamics of the peak response (priming); long-term protection requires the persistence of antibodies and the generation of immune memory cells capable of rapid and effective reactivation [24].

8 Immune Memory

As illustrated, T-helper lymphocytes play an important role in the regulation of both T and B cell responses as well as cytotoxic T-lymphocytes. However, the most important property of adaptive immunity is its capacity to establish an immunological memory response, assuring a stronger and faster protective immune response whenever challenged again by the same pathogen. While the primary immune response on average takes 10–14 days to build up, immunological memory shortens the immunological reaction time to a couple of days, thereby effectively preventing future reinfection with the same agent (Fig. 6).

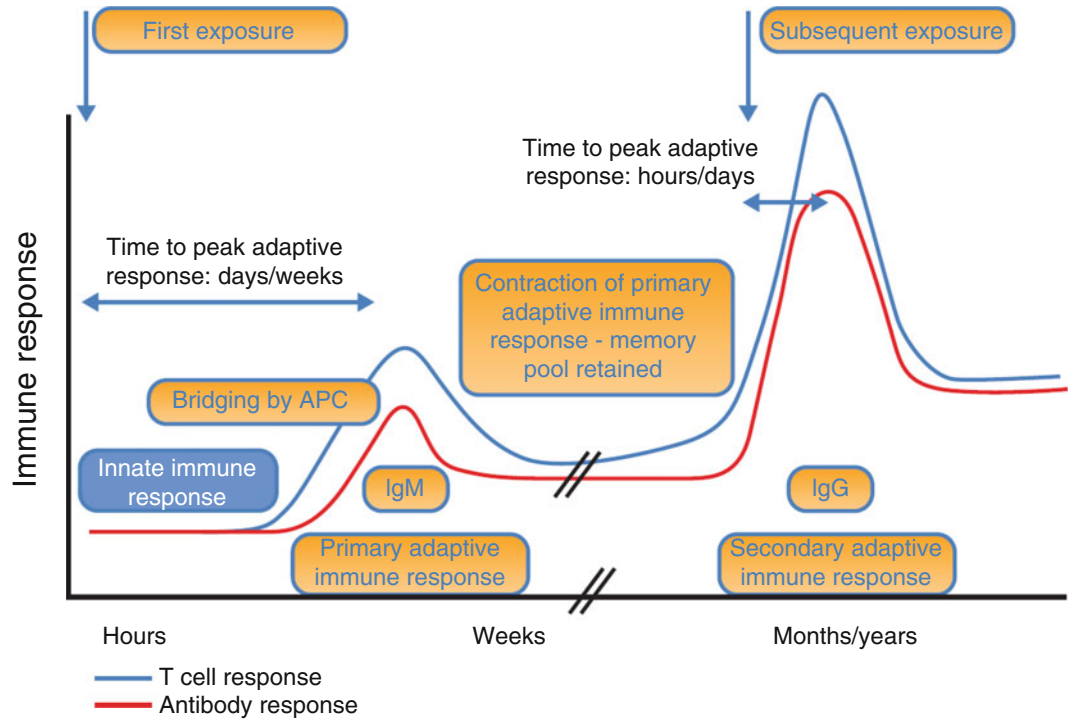

Fig. 6 Dynamics of the adaptive immune response. Adapted from "Understanding Modern Vaccines: Perspectives in Vaccinology, Volume 1", 2011 Elsevier, Oberdan, L., Cunningham, A., Stern, P.L.: Chapter2. Vaccine immunology; p. 45

At the first encounter with an antigen usually only a small number of lymphocytes expressing a given antigen specificity are available. Upon activation by antigen recognition, T and B lymphocytes will go through rapid proliferation, leading to the accumulation of an increased number of cells expressing receptors for the specific antigen. Some of these cells will differentiate into effector cells while others will become "memory cells," able to survive for longer periods of time within the host. Any exposure to an antigen (pathogen or vaccine) therefore leads to a long-term modification of the cellular repertoire, such that the relative frequency of T and B cells specific for an individual antigen is increased in antigen-exposed individuals compared with naïve individuals [27, 28]. Memory T and B cells will develop secondary (recall) responses on reencounter with their specific antigen. The adaptive response on secondary exposure leads to a rapid expansion and differentiation of memory T and B cells into effector cells, and the production of high levels of antibodies. A higher proportion of IgG and other isotypes of antibodies compared with the level of IgM characterizes memory antibody responses. During the process of reactivation the binding avidity of antibodies can be optimized by somatic hypermutation of the variable antigen-binding region.

The capacity to generate immune memory is the key feature of the adaptive immune system and is crucial for maintenance of long-term protection. This capacity to establish an immunological memory response also is the fundamental basis for the biological effects of vaccines. Initially antigen processing and presentation by dendritic cells (DCs) are key steps that define the environment and the course of efficient immune responses [6]. Therefore, innate immunity sets the scene for the subsequent adaptive response and innate and adaptive immunity have to interact vigorously in order to initiate the most effective type of protective immunity.

9 How Do Vaccines Mediate Protection?

Long-term protection is ensured by the maintenance of antigen-specific effector cells and/or by the induction of immune memory cells that can be rapidly reactivated into immune effectors whenever the organism is challenged with the same pathogen again in the future. Vaccine-induced immune effectors are essentially antigen-specific antibodies produced by plasma cells that are capable of binding specifically to a toxin or a pathogen. Other effectors are cytotoxic CD8+ T cells that can limit the spread of infectious microorganisms by killing infected cells or secreting specific antiviral cytokines. The generation and maintenance of both B and CD8+ T cell responses are supported by growth factors and signals provided by CD4+ T helper cells. Most antigens and vaccines trigger both B and T cell responses. In addition,

CD4+ T cells are required for most antibody responses, while antibodies exert significant influences on T cell responses to intracellular pathogens.

10 Immune Correlates of Protection

Ideally a successful immune response is measured by the quality of the acquired protection from infection; however, this approach usually is difficult to perform regularly on individual basis. Alternatively the emerging immune response may also be assessed by detection of antigen-specific antibodies or a particular pattern of cytokine expression by T cells. These surrogate markers or correlates of protection can only be defined based on clinical trials where protection from disease or infection is determined in cohorts of vaccinated versus unvaccinated individuals [29].

The majority of vaccines developed so far have been assessed only by their ability to elicit antigen-specific antibody responses (Table 2). However, while detection of specific antibodies in principle illustrate vaccine-related immune responses, protective antibody titers/concentrations have been defined only for a small number of vaccinations. For example in the case of rubella protective antibody titers can be reliably assessed to determine whether an individual is protected post-vaccination. However, most immune correlates of protection are not well defined. Historically, demonstration of the production of specific antibodies has been the main goal of vaccination; however, this concept appears to be insufficient or inappropriate for future vaccine development.

11 Principles of Vaccine Development

During the interaction with an infectious agent, the immune system develops and optimizes an effective defense strategy that prevents further spread of the pathogen, interrupts its life cycle, and eventually eliminates it from the body. Thereafter, the affected individual ideally acquires protective immunity that prevents the recurrence of an infection by the same agent in the future. In order to provide protection from infectious diseases vaccines have to be designed to induce immune responses comparable to the natural occurring immune response against an infectious agent. However, there is a significant difference between the expected effects of vaccines and those that are attributed to infectious agents. While it is common knowledge that infections are usually associated with clinical symptoms of disease, such a coincidence generally is not acceptable for the use of vaccines.

Table 2
Accepted immunological correlates of protection. Adapted from Plotkin SA. Correlates of protection induced by vaccination. Clinical and Vaccine Immunology; 2010; 17:1055–1065

Disease	Vaccine	Protective AB concentration/titer
Diphtheria toxoid	Toxoid	≥0.01–0.1 IU/ml
Hepatitis A	Killed	>10 IU/L
Hepatitis B (HBsAg)	Protein	>10 IU/L
Hib PS	Polysaccharide	1 μg/ml
Hib glycoconjugates	Polysaccharide-protein	>0.15 mg/L
Influenza	Killed, subunit	No correlate
Influenza intranasal	Live attenuated	No correlate
Measles	Live attenuated	≥1:2 (HHT) or ≥) 200 mIU/ml Microneutralization (EIA)
Meningococcal PS	Polysaccharide	≥1:4, Human complement bactericidy-assay
Meningococcal conjugates	Polysaccharide-protein	≥1:4, Human complement bactericidy-assay
Mumps	Live attenuated	No correlate
Papillomavirus	Viruslike particle	No correlate
Pertussis, whole cell	Killed	No correlate
Pertussis, acellular	Protein	No correlate
Pneumococcal PS	Polysaccharide	0.2 to >0.35 μg/ml depending on serotype
Pneumococcal conjugates	Polysaccharide-protein	0.2 to >0.35 μg/ml for infants depending on serotype, ELISA; Titer 1:8; opsonophagocytosis
Polio Sabin	Live attenuated	Titer≥ 1:4 to 1:8
Polio Salk	Killed	≥0.075 IU/ml Polio type 1 ≥0.18 IU/ml Polio type 2 ≥0.08 IU/ml Polio type 3
Rabies	Killed	>0.5 IE/ml or titer ≥ 1:16
Rotavirus	Live attenuated	No correlate
Rubella	Live attenuated	10–15 mIU/ml; immunoprecipitation assay
Tetanus toxoid	Toxoid	≥0.1 IU/ml
Tuberculosis (BCG)	Live mycobacterium	>0.5 IE/ml or ≥Titer 1:16
Varicella	Live attenuated	>5 gp ELISA-Units/ml
Yellow fever	Live attenuated	Titer 1:5, neutralization assay

Symptoms of an infection are either caused directly by the pathogen, or, more often, they are consequences of the emerging immune response, representing side effects of our physiological defense mechanisms. Typical complaints such as physical discomfort, malaise, fever, or organ malfunction in most cases are related to inflammatory reactions that occur in course of the immunological defense process. Since vaccines are administered to prevent infections and/or diseases, they are expected to provide protection without the risk of side effects or clinical symptoms of disease. To this end in vaccine development it is important to understand the life cycle of an infectious agent, how it multiplies and infests the human organism, and how the immune system counteracts and overcomes the microbial invasion and finally builds up a protective immunity, i.e., an effective barrier against future challenges by the same agent. Moreover, it is essential to define which elements of the natural immune response are relevant for the elimination of the pathogen and future protection, and which are responsible for symptoms of disease and discomfort. Ideally, a vaccine should induce only the elements of the natural immune response that are essential for protection, but simultaneously exclude all negative effects of natural infection. In vaccine development, therefore, not only the elements of the immune response guaranteeing best protection must be considered, but also the acceptable tolerability and safety ramifications of the induced inflammatory response. As a consequence the design of a vaccine has to be based on both structural and biological properties/qualities of an infectious agent as well as the type and quality of naturally occurring immune responses initiated by the infectious pathogen.

Initially vaccine development focused on the steps required to elicit activation of a protective immunity and generation of immunological memory by virtually mimicking the interaction of an infectious agent with the human immune system without posing any risks of the infectious disease to the vaccinee. This requires the identification of antigenic structures relevant for protection as well as definition of immune response mechanisms adequate to elicit protective immunity. The latter will vary according to specific disease (Table 3). While for many decades vaccine development concentrated primarily on targeting components of the adaptive immunity (B cells or immunoglobulins, T cells, and cytokines, such as interferon), recent research indicates that innate and adaptive immunity have to interact vigorously to initiate the most potent type of protective immune response [16]. In particular, antigen processing and presentation by DCs are key steps in the development of efficient immune responses. The recognition of the important role of innate immunity in controlling the adaptive response (Figure 7) has led to a reappraisal of the role of adjuvants in vaccinology [16].

Table 3
Quality of protective immune responses to infection differs according to type of disease

Disease	Pathogenesis	Protective immune response
Cholera	Local infection, enterotoxins	IgA (mucosa) IgG (neutralization)
Diphtheria	Local infection, exotoxins	IgG (neutralization of toxin)
Hepatitis B	Systemic infection	IgG (elimination of the virus) IFNγ, T cells (infected cells)
Human papillomavirus	Local infection	IFNγ, IgG (neutralization) T cells (lysis of infected cells)
Influenza	Local infection Systemic infection	IFNγ, IgA, IgG T cells (infected cells)
Poliomyelitis	Systemic infection	IgA (elimination of the virus) IFNγ, T cells (infected cells)
Tuberculosis	Systemic infection	T cells, IFNγ, killing of intracellular pathogen by macrophages

Ig immunoglobulin, *IFN* interferon

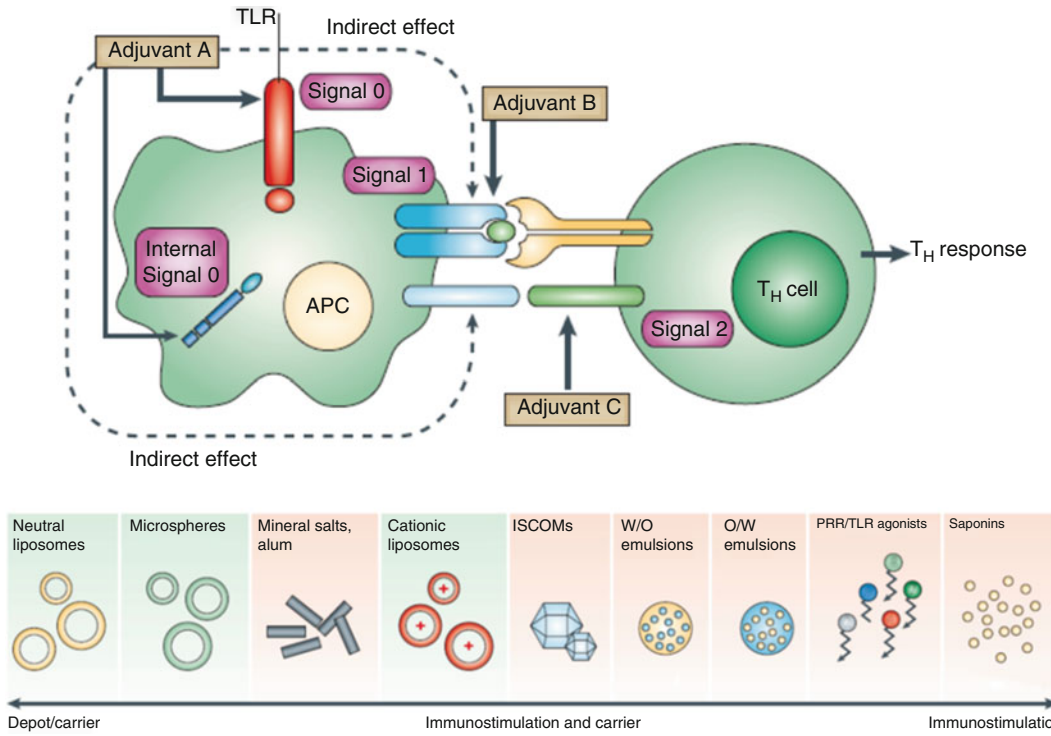

Fig. 7 Role of adjuvants in vaccinology. Adapted from Guy B: The perfect mix: recent progress in adjuvant research. Nat Rev Microbiol. 2007 Jul;5(7):505–17

In most instances, vaccines are developed to protect human beings from infectious diseases on a population-based level. This implies that vaccines should provide protection for basically every vaccinated individual within an immunogenetically heterogeneous population. Conventional vaccines formulated with whole microbial pathogens usually provide a broad range of different antigens and antigenic epitopes that in most instances guarantee sufficient immunostimulatory activity for a heterogeneous population. In contrast, highly purified antigens consisting only of a limited number of epitopes may pose the risk of insufficient interaction with individuals missing the adequate immune receptor repertoire. Moreover, genetic heterogeneity of the pathogen may counteract the expected benefit of highly purified vaccine antigens. Keeping this in mind, selection of vaccine antigens has to balance specificity and purity of antigens against sufficient antigenic variety to ensure targeting the immune system of every or at least the majority of individuals in a given population.

12 Selecting Vaccine Antigens

The identification of appropriate antigenic structures involves various considerations, based on the desired type of immune response. For example, if a neutralizing antibody response is sufficient to protect from infection, usually an antigenic structure from the bacterial/viral cell surface is selected. This has been done successfully for the *H. influenzae* type b, pneumococcal and meningococcal and hepatitis B vaccines, or from secreted toxins, like tetanus or diphtheria.

In the course of an antibody response, antigen-specific helper T cells are essential for the evolution of high-affinity antibodies and immune memory. Other antigen-specific T cells, including cytotoxic T cells, accomplish important effector functions, such as the targeted removal of host cells infected by intracellular pathogens, or support for macrophages in their removal of extracellular pathogens. In these latter cases an antigen has to be selected for the vaccine that enables these T cell effector-mediated responses. Hepatitis B vaccines, for example, induce antibodies as well as hepatitis B-specific T cell responses [30], pertussis vaccines induce antibodies and stimulate helper T cells to produce interferon [31, 32], and hepatitis A and IPV vaccines probably stimulate both T and B cells. As a matter of fact, in some instances the immune response induced by vaccination may even be stronger than the response observed after natural infection. This has been observed for human papillomavirus (HPV) vaccines that induce higher concentrations of neutralizing antibodies than in naturally occurring immune responses [33].

Purification of vaccine antigens is an important step to achieve vaccines with few unwanted side effects. Progress in biotechnology

in recent years has allowed isolating subcomponents of pathogens and producing them in large quantities. By eliminating unwanted pathogenic components, the high specificity and purity of these antigens permit the development of vaccines with reduced reactogenicity and improved safety profiles. The first attempt to select antigenic structures and to eliminate unwanted material has been made with split- or subvirion vaccines. These vaccines are prepared by using a solvent (such as ether or a detergent) to dissolve or disrupt the viral lipid envelope [7]. The technology has been applied most successfully in the development of inactivated influenza vaccines [34]. Purification steps are also engaged in the production of subunit vaccines, comprising protein or polysaccharide antigens, such as acellular pertussis proteins [6], typhoid Vi-antigen, and pneumococci polysaccharides [35, 36]. While split and subunit vaccines are less reactogenic than their conventional whole-cell counterparts, in many instances, this benefit is associated with reduced immunogenicity. For these vaccines the addition of adjuvants (*see* below) often is required to induce sufficient immunological memory and maintain protection [16].

Impaired immunogenicity may also occur with purified antigens that are unable to address sufficient elements of the immune system relevant for the protective response. Immune responses to pure polysaccharide antigens can be particularly poor in comparison with those induced by protein antigens. Polysaccharide antigens alone are not able to recruit T-helper cells in order to obtain B cell support by cell-mediated immunity. This phenomenon is especially significant in young infants and children as well as with the elderly [37]. As a result, immune responses to plain polysaccharide antigens are characterized by the secretion of low-affinity antibodies, mainly immunoglobulin M (IgM) molecules, and display a stereotyped "innate response" behavior. Repetitive encounters with the same antigen fail to induce a secondary, memory-like immune response [16]. This disadvantage was finally surmounted by the invention of the protein-conjugate technology. By covalently binding the polysaccharide antigen to a carrier protein, typically an inactivated toxoid like tetanus or diphtheria toxoid, conjugate vaccines dramatically improve immune responses to polysaccharides. With these vaccines, the polysaccharide component is recognized and bound by the B cell antigen receptor (i.e., the antibody molecule expressed on the cell surface), providing the first signal for B cell activation. Subsequently, the responding B cell serves as an antigen-presenting cell for T-helper cells that are specific for the conjugated carrier protein. The conjugated vaccine is internalized and processed and the antigen components of the conjugated protein are presented in the context of MHC molecules to be recognized by conjugate-protein/peptide-specific T-helper cells. Applying this approach, polysaccharide-specific B cells recruit help from conjugate-protein-specific T cells to

get all signals needed to promote further activation as well as isotype switching to IgG production and generation of memory B cells. Today, this elegant technique is regularly applied to vaccines containing bacterial polysaccharides for the prevention of invasive diseases caused by encapsulated bacteria. Examples include *H. influenzae* type b, pneumococcal [36] and meningococcal vaccines.

Modern molecular biology techniques allow vaccinologists today to select antigenic structures at the gene level and produce recombinant vaccines that contain only the antigen substructures relevant to elicit protective immunity [38]. The first recombinant vaccine, licensed in 1986, was achieved by cloning the gene for hepatitis B surface antigen (HBsAg) and was as effective as plasma-derived vaccines [39]. Two recombinant vaccines are also available against cervical cancer [40]. Both vaccines are based on HPV viruslike particles assembled from recombinant HPV L1 coat proteins. Quite recently research in this area has taken even a step further. By expressing multiple proteins identified in genome of meningococcus type B strains it was possible to identify new protein antigens on the surface of the microorganism that finally led to the successful development of a MenB vaccine. This technology, named reverse vaccinology, is now engaged to develop vaccines against microorganisms for which hitherto no vaccines were available, such as vaccines against *Staphylococcus aureus* or *Pseudomonas* strains.

However, as with subunit vaccines, the highly purified antigens obtained with peptide and recombinant technologies can have the disadvantage of weakened immunogenicity. The research for means to overcome this shortcoming has led to the development of innovative adjuvants to control and modify vaccine-induced immune responses, as described in the next section.

13 Improving Vaccines over Natural Immune Responses

Over the last century the approach to vaccine design has moved from vaccines (many of them still available today) that were developed empirically to the development of vaccines with higher specificity, better activation of relevant immunological mechanisms, lower reactogenicity, and better safety profiles. With these advances, a major challenge emerged; in comparison to whole inactivated microorganisms or less purified vaccines highly purified and defined antigens can have the unwanted consequence of impaired immunogenicity. While live attenuated or killed whole organisms contain a multitude of antigenic structures that can act as “intrinsic adjuvants” [4] to enhance their immunogenicity, this quality often is lost with the purification process of subunit vaccines. In order to

Table 4
Adjuvants potentiate vaccine-induced immune responses

• Prolonged antigen persistence
• Kinetics of lymphocyte trafficking
• Antigen processing and presentation
• Modulation of immune responses
– Th1–Th2 activation
– Cytokine production
– Augmentation of AB production
– Induction of "mucosa immunity"

conserve the advantages of subunit vaccines it was necessary to develop tools, i.e., adjuvants that support a sufficient activation of the immune system (Table 4).

Traditional vaccine adjuvants include aluminum salts, emulsions, and liposomes [4]. Aluminum salts have been used widely as adjuvants in human vaccines for more than 80 years [41]. It is now known that aluminum salts (and other adjuvants) are able to provide proinflammatory or immunostimulatory effects as well as prolong the persistence of vaccine antigens by slowing down antigen degradation. However, it has also been demonstrated that aluminum salts primarily promote antibody responses, with little or no effect on T-helper 1 and cytotoxic T cell immune responses, which are key for protection against many pathogens [16]. Oil-in-water (O/W) emulsions have a good safety profile and are capable of eliciting a strong humoral response. An example is MF59™, which is composed of stable droplets of the metabolizable oil squalene, and two surfactants, polyoxyethylene sorbitan monooleate (Tween 80) and sorbitan trioleate (Span-85). Enhancement of the immune response generated by MF59™ appears to be limited to antibody responses [35].

Especially for vaccines designed to induce a cytotoxic T cell-mediated immune responses, aluminum salts have been found to be inadequate to imitate the required protection. This is essentially due to a lack of "intrinsic immune defense triggers" usually provided by the pathogen, such as pathogen-associated molecular patterns (PAMPs) [16]. Naturally available PAMPs may be reduced or even become lost during the selection process for relevant vaccine antigens or in the course of the purification process. PAMPs represent conserved "danger signals" that are recognized by pattern recognition receptors (PRRs), mainly of the innate immune system and to some degree also on B and T cells, including so-called Toll-like receptors (TLRs). The targeting of PRRs by

PAMPs delivers an important early activation signal that can alert and potentiate multiple aspects of the adaptive immune responses, i.e., type, magnitude, and quality of specific B and T cell activation, and immune memory induction. Hence, it is by the recognition of particular PAMPs the innate immune system can create different immunological environments that can shape the type of protective adaptive immune responses. It was a logical step in vaccine development to target the "danger-sensing" PRRs in order to improve the quality and persistence of vaccine-related immune responses. A variety of TLR agonists have also been identified as potential vaccine immunomodulators, including deacylated monophosphoryl lipid A (MPL) [42], a purified, detoxified derivative of the lipopolysaccharide (LPS) molecule of the bacterial wall of *Salmonella minnesota* [43]. Like LPS, MPL acts through binding to TLRs, stimulating upregulation of co-stimulatory molecules and cytokine release, inducing a strong humoral and cellular response, depending on the antigen considered [55]. Most recently, the improved understanding of TLR signaling has led to the recognition of a role for immunostimulatory DNA, such as CpG [44], and other TLR agonists, such as messenger RNA molecules as vaccine adjuvants.

Other molecules besides TLR agonists have also been identified as immunomodulators and are currently investigated as vaccine adjuvants. For example, QS-21 is a highly purified immunostimulant extracted from the bark of the South American tree *Quillaja saponaria* [45]. It has the ability to optimize antigen presentation to antigen-presenting cells and stimulate both humoral and cellular responses. Importantly, the adjuvant properties of MPL and QS-21 appear synergistic. MPL and QS-21 have been studied in combination and have been shown to enhance Th1 and cytotoxic T cell responses against exogenous protein in mice.

Liposomes are synthetic nanospheres consisting of lipid layers that can encapsulate antigens and act as both a vaccine delivery vehicle and an adjuvant [46]. Liposomes promote humoral and cell-mediated immune responses to a wide range of bacterial and viral antigens as well as tumor cell antigens. Vaccines containing liposomes are available against hepatitis A and influenza [47].

Adjuvant research has demonstrated that with the right selection of antigens together with new adjuvants the immune response elicited by vaccines can be adapted to the pathogens and targeted populations. Recognizing that this cannot always be achieved with only one adjuvant type led to investigation of adjuvant systems, which combine classical adjuvants (aluminum salts, o/w emulsion, and liposomes) and immunomodulatory molecules, such as MPL and Q-S21. This concept has allowed the development of vaccines tailored to the antigen and target population, such as the HPV vaccine with adjuvant system AS04 and a malaria vaccine with adjuvant system AS02.

14 Future Prospects

Some challenges presented by infectious diseases such as malaria, tuberculosis, and HIV/AIDS so far could not be addressed successfully with classical vaccines, including those containing traditional adjuvants. This has led to new approaches including live vectors, DNA vaccines, and new adjuvant formulations as described before. Live vector technology involves the use of attenuated bacteria and viruses as vectors for the delivery of pathogen-specific DNA to enhance immunogenicity [48, 49]. The technology is of particular interest for the development of HIV vaccines and therapeutic vaccines for certain cancers. However, to date, clinical trials performed in the context of HIV have been disappointing, and the potential of such an approach is unclear.

DNA vaccines are composed of genes encoding a key antigenic determinant, often inserted into a bacterial plasmid [50]. Administration of the DNA vaccine leads to the expression of the foreign gene and synthesis of antigens derived from the infectious organism within the host cells. Presentation of the foreign proteins by the host cells can elicit an immune response similar to that induced by natural infection. Depending on the host cells targeted, DNA vaccines have the potential to stimulate a cellular immune response and, to a lesser extent, a humoral immune response. Since the foreign protein produced is expressed and processed intracellularly it can also be presented to the immune system in the context of the MHC class I system, providing the option to stimulate specific cytotoxic effector T cells. In contrast, traditional vaccines are mostly processed via the MHC class II system and therefore will preferentially stimulate T-helper lymphocytes. Clinical trials of plasmid DNA vaccines for HIV infection, Ebola hemorrhagic fever, West Nile virus infection, avian influenza, and various cancers are currently ongoing.

Many more advances can be expected in future vaccinology, not only against infectious diseases but also against other illnesses or chronic disorders not necessarily associated with an infectious pathogen. These include therapeutic cancer vaccines that have been tested with promising results with a number of spontaneous tumor animal models, including models of breast, prostate, pancreatic, and colon cancer [51]. These vaccines are designed involving antigen-specific vaccines and DC vaccines formulated with patients' DCs loaded with tumor-associated antigens. Moreover, cytokines are being evaluated as cancer vaccine adjuvants, most notably granulocyte macrophage colony-stimulating factor (GM-CSF). DC vaccines and antigen-specific cancer immunotherapeutics (ASCI) represent the most advanced approaches in cancer immunotherapy. DC vaccines work by isolating and exposing the cancer patient's DCs ex vivo to compounds that include

tumor-associated antigens. After their reintroduction to the patient, these DCs promote a cytotoxic T cell response against the tumor tissue.

Allergic diseases affect up to 25 % of the population in Western countries. Novel immunotherapies are currently under development, among them a vaccine including a TLR-9 agonist [52]. A phase 2 study with a ragweed allergen conjugated to immunostimulatory DNA (CpG) showed reduction in symptoms of allergic rhinitis during the ragweed season [53], but further studies are needed to confirm these observations. Progress is also expected from vaccines for the treatment of autoimmune diseases like type 1 diabetes, arthritis, Alzheimer's disease, or multiple sclerosis. Again encouraging results with DNA vaccines have been found in phase 1/2 studies for multiple sclerosis and type 1 diabetes [54]. The continuing progress in vaccine technologies and in the understanding of the mechanisms underlying the immune response is facilitating a more and more refined approach to vaccine design tailored to the desired effect of combating disease.

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