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INTRODUCTION

Since vaccination was documented by Edward Jenner in 1798, it has become the most successful means of preventing infectious diseases, saving millions of lives every year. However, application of vaccines is currently not limited to the prevention of infectious diseases. Vaccines in the pipeline include anti-drug abuse vaccines (nicotine, cocaine) and vaccines against allergies, cancer, and Alzheimer's disease.

Modern biotechnology has an enormous impact on current vaccine development. The elucidation of the molecular structures of pathogens and the tremendous progress made in immunology as well as developments in proteomics and bioinformatics have led to the identification of protective antigens and ways to deliver them. Together with technological advances, this has caused a move from empirical vaccine development to more rational approaches. A major goal of modern vaccine technology is to fulfill all requirements of the ideal vaccine as summarized in Fig. 22.1, by expressing antigen epitopes (= the smallest molecular structures recognized by

the immune system) and/or isolating those antigens that confer an effective immune response and eliminating structures that cause deleterious effects. Thus, better-defined products can be obtained, resulting in improved safety. In addition, modern methodologies may provide simpler production processes for selected vaccine components.

In the following section, immunological principles that are important for vaccine design are summarized. Subsequently, classical vaccines which are not a result of modern genetic or chemical engineering technologies will be addressed. Classical and modern vaccines are listed in Table 22.1. Current strategies used in the development and manufacture of new vaccines are discussed in the section "Modern Vaccine Technologies." It is not our intent to provide a comprehensive review. Rather, we will explain modern approaches to vaccine development and illustrate these approaches with representative examples. In the last section, pharmaceutical aspects of vaccines are dealt with.

The ideal vaccine

- Is 100 % efficient in all individuals of any age
- Provides lifelong protection after single administration
- Does not evoke an adverse reaction
- Is stable under various conditions (temperature, light, transportation)
- Is easy to administer, preferably orally
- Is available in unlimited quantities
- Is cheap

Figure 22.1 ■ Characteristics of the (hypothetical) ideal vaccine.

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Category	Technology	Live/nonliving	Characteristics
Attenuated vaccines	Classical	Live	Bacteria or viruses attenuated in culture; empirically developed
Inactivated vaccines	Classical	Nonliving	Heat-inactivated or chemically inactivated bacteria or viruses; empirically developed
Subunit vaccines	Classical	Nonliving	Extracts of pathogens; combination of purified proteins with killed suspension; purified single components (proteins, polysaccharides); combination of purified components with adjuvant; purified components in a suitable presentation form; polysaccharide-protein conjugates
Genetically improved live vaccines	Modern	Live	Genetically attenuated microorganisms; live viral or bacterial vectors
Genetically improved subunit vaccines	Modern	Nonliving	Proteins expressed in host cells; recombinant protein/peptide vaccines
Recombinant subunit vaccines identified by reverse vaccinology	Modern	Nonliving	Recombinant antigenic proteins obtained from the genomic sequence of the pathogen
Synthetic peptide-based vaccines	Modern	Nonliving	Linear or cyclic peptides; multiple antigen peptides; peptide-protein conjugates
Nucleic acid-based vaccines	Modern	Nonliving	DNA or mRNA coding for antigen

Table 22.1 ■ Categories of classical vaccines and vaccines obtained by modern technologies.

IMMUNOLOGICAL PRINCIPLES

■ Introduction

After a natural infection, the human immune system in most cases launches an immunological response to the particular pathogen. After recovery from the disease, the immunological response indeed protects the affected individual from that disease, in the ideal case forever. This phenomenon is called specific immunity and is caused by the presence of circulating antibodies, cytotoxic cells, and memory cells. Memory cells become active when the same type of antigenic material enters the body on a later occasion. Unlike the primary response after the first infection, the response after repeated infection is very fast and usually sufficiently strong to prevent reoccurrence of the disease.

The principle of vaccination is mimicking an infection in such a way that the natural specific defense mechanism of the host against the pathogen will be activated, but the host will remain free of the disease that normally results from a natural infection. This is effectuated by administration of antigenic components that consist of, are derived from, or are related to the pathogen. The success of vaccination relies on the induction of a protective immune response and a long-lasting immunological memory. Vaccination is also referred to as active immunization, because the host's immune system is activated to respond to the "infection" through humoral and cellular immune responses, resulting in adaptive immunity against the particular pathogen. The immune response is generally highly specific: it discriminates not only between pathogen

species but often also between different strains within one species (e.g., strains of meningococci, poliovirus, influenza virus). Albeit sometimes a hurdle for vaccine developers, this high specificity of the immune system allows an almost perfect balance between response to foreign antigens and tolerance with respect to self-antigens. Apart from active immunization, administration of specific antibodies can be utilized for short-lived immunological protection of the host. This is termed passive immunization (Fig. 22.2).

Traditionally, active immunization has mainly served to prevent infectious diseases, whereas passive immunization has been applied for both prevention and therapy of infectious diseases. Through recent developments new potential applications of vaccines for active immunization have emerged, such as the prevention of other diseases than infectious diseases (e.g., cancer) and for the treatment of substance abuse (e.g., nicotine addiction). Such vaccines are referred to as therapeutic vaccines. The difference between passive and active immunization is outlined in Fig. 22.2. Since antibody preparations for passive immunization do not fall under the strict definition of a vaccine, they are not further discussed here.

■ Active Immunization: Generation of an Immune Response

The generation of an immune reaction against a pathogen by vaccination follows several distinct steps that should ultimately lead to long-lasting protection against the pathogen through memory cells. These steps are

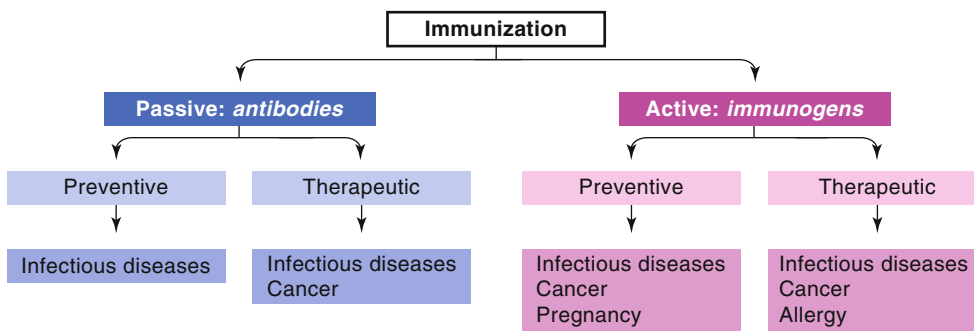


Figure 22.2 ■ Scheme of active immunization (= vaccination) and passive immunization and examples of their fields of application.

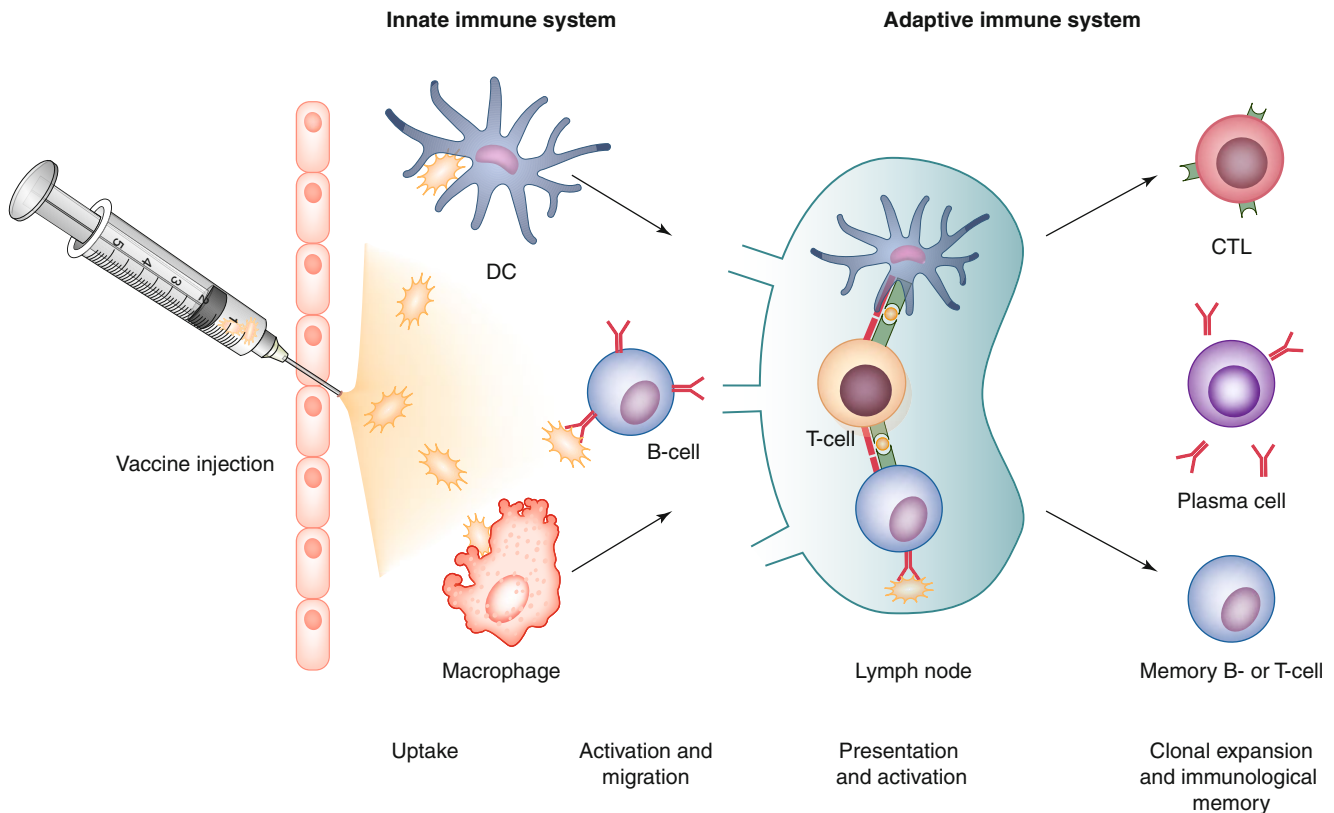


Figure 22.3 ■ Overview of the steps leading to immunization after administration of a vaccine. Upon subcutaneous or intramuscular administration, the vaccine components are taken up by phagocytic cells such as macrophages and dendritic cells (DCs) that reside in the peripheral tissue and express pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs). Professional antigen-presenting cells (APCs) that have taken up antigens become activated and start migrating towards nearby lymph nodes. Inside the lymph nodes, the antigen processed by the APCs is presented to lymphocytes, which, when recognizing the antigen and receiving the appropriate co-stimulatory signals, become activated. These antigen-specific B and T lymphocytes clonally expand to produce multiple progenitors recognizing the same antigen. In addition, memory B and T cells are formed that provide long-term (sometimes lifelong) protection against infection with the pathogen.

uptake of the vaccine (consisting of either the entire pathogen or antigenic components thereof) by phagocytic cells, activation and migration of professional antigen-presenting cells (APCs) from infected tissue to peripheral lymphoid organs, antigen presentation to T lymphocytes, and finally activation (or inhibition) of T and B lymphocytes. The entire process is illustrated in Fig. 22.3. Below we describe the successive steps leading

to an immune response to a pathogen, which are important for the design of vaccines against the pathogen.

■ Innate Response

Every immune reaction against a pathogen starts with activation of the innate immune system. This is a nonspecific fast response against antigens. Important constituents of the innate system are antigen sampling

PRR	PAMP
TLR-1 TLR-2	Triacyl lipoproteins Peptidoglycans Lipoproteins Lipoarabinomannan Zymosan
TLR-3	Viral dsRNA
TLR-4	Lipopolysaccharide, lipid A, Taxol
TLR-5	Flagellin
TLR-6	Diacyl lipoproteins
TLR-7	Small, synthetic compounds, ssRNA
TLR-8	Small, synthetic compounds
TLR-9	Unmethylated CpG DNA
TLR-10	Unknown
TLR-11	Components from uropathogenic bacteria
Scavenger receptors	Polyanionic ligands
C-type lectin receptors	Sulfated sugars and mannose-, fucose-, and galactose-modified polysaccharides and proteins
NOD-1, NOD-2	Peptidoglycans
Type 3 complement receptors	Zymosan particles, β -glucan

Adapted from Pashine et al. (2005)

Table 22.2 ■ Examples of pattern recognition receptors (PRRs) and their ligands (PAMPs).

cells like macrophages and dendritic cells (DCs). The innate response does not lead to immunological memory. Phagocytic cells sense conserved microbial structures called pathogen-associated molecular patterns (PAMPs). They do this via pattern recognition receptors (PRRs) on the cell surface (for bacterial PAMPs) or in the cytoplasm (for viral PAMPs). Examples of PRRs are toll-like receptors (TLRs), scavenger receptors, and C-type lectins (Table 22.2). TLRs consist of a family of receptors, with each member recognizing different patterns of pathogens (Kawai and Akira 2010). TLRs can be found on many cells including macrophages and DCs. DCs can also engulf materials from their extracellular environment by a receptor-independent process called pinocytosis.

■ Activation and Migration

Besides mediating uptake of antigenic material from the surrounding tissue, PRRs also play an important role in triggering the cytokine network that will eventually influence the type of adaptive immune response that will be evoked against the pathogen. The phagocytic cells that have taken up pathogens from the infected tissue become activated and start to produce proinflammatory cytokines such as interleukin-1 β ,

interleukin-6, and tumor necrosis factor- α as well as chemokines. The chemokines recruit more phagocytic cells such as neutrophils and monocytes to the infection site, whereas the proinflammatory cytokines induce fever and the production of acute-phase response proteins that can opsonize pathogens.

Most phagocytic cells, including DCs and macrophages, and also B cells can serve as APCs to present processed antigenic determinants to lymphocytes in the peripheral lymphoid organs. For instance, DCs that have taken up antigens from infected tissue become activated and migrate via the afferent lymphatic vessels towards nearby lymph nodes where the encounter with pathogen-specific lymphocytes can take place.

■ Antigen Presentation and Lymphocyte Activation

The peripheral lymphoid organs are the primary meeting place between cells of the innate immune system (APCs) and cells of the adaptive immune system (T cells and B cells). Upon interaction with APCs, pathogen-specific T cells and B cells will be activated, provided that they acquire the appropriate signals from the APCs. Besides antigen-specific binding via their antigen receptors, lymphocytes require co-stimulatory signals via interaction of accessory and co-stimulatory molecules between lymphocytes and APCs. This cell-cell interaction is essential for proper stimulation of lymphocytes, and without those accessory signals, antigen-specific T cells may become anergic. Lymphocytes receiving the appropriate signals for activation will clonally expand and generate multiple progenitors all recognizing the same antigen. Clonal expansion is a typical feature of the adaptive immune system, which will be discussed in more detail below.

■ The Adaptive Immune System

The adaptive immune system is involved in elimination of pathogens in the late phase of infection and in the generation of immunological memory. It comprises B and T lymphocytes both bearing antigen-specific receptors. The adaptive immune system can be divided into humoral immunity and cell-mediated immunity (CMI) (see Fig. 22.4 and Table 22.3). The humoral response results in antibody formation (but contains cell-mediated events, see Fig. 22.4, panels a, b); CMI results in the generation of cytotoxic cells (see Fig. 22.4, panels a, c). The action of antibodies and T cells is dependent on accessory factors, some of which are mentioned in Table 22.3. In general, after infection with a pathogen or a protective vaccine, both humoral and cellular responses are generated. This indicates that both are needed for efficient protection. The balance between humoral and cellular responses, however, can differ widely between pathogens and is dependent on how the pathogen is presented to the adaptive immune

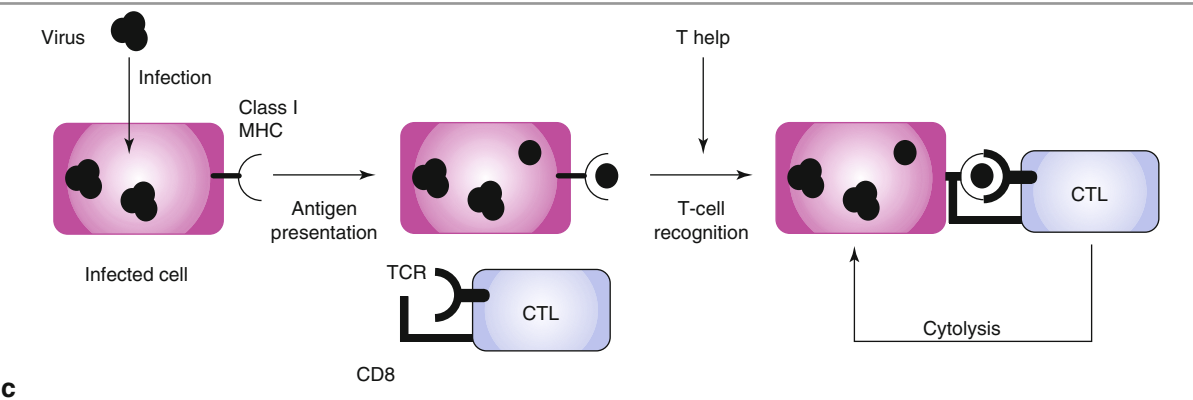
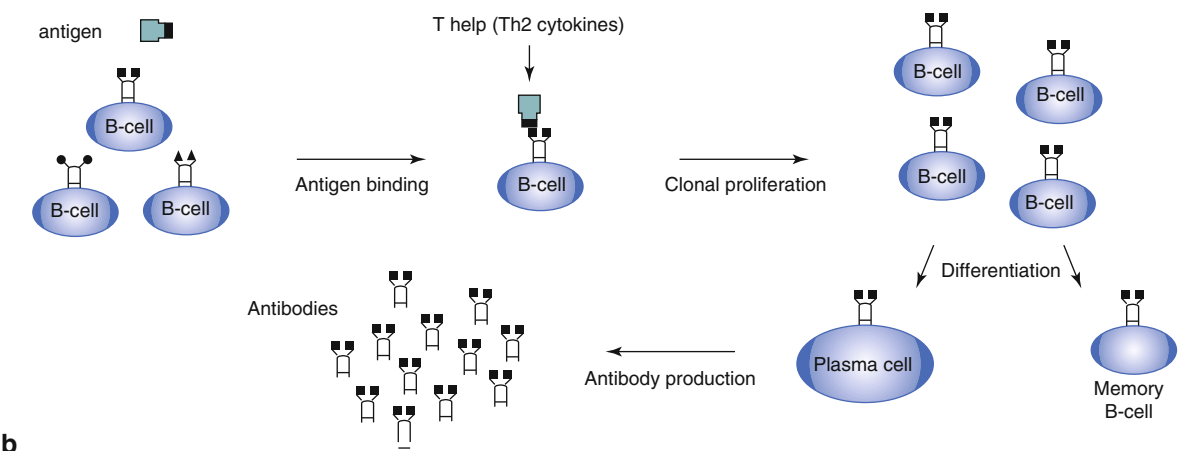
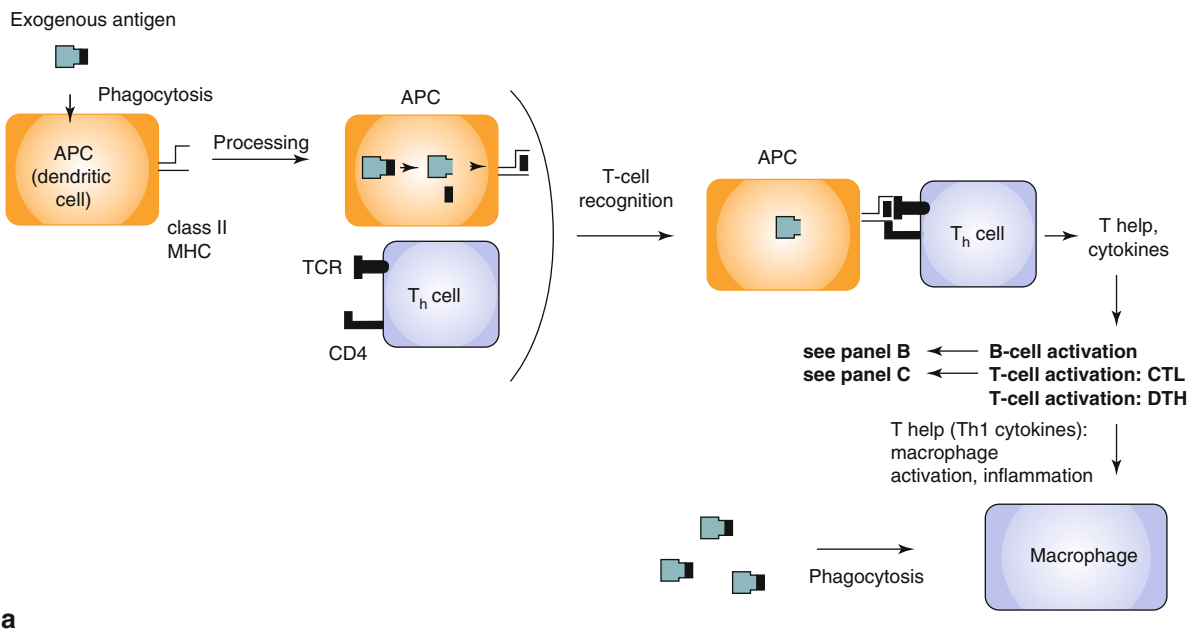


Figure 22.4 ■ Schematic representation of antigen-dependent immune responses. **(a)** Activation of T-helper cells (Th-cells). An antigen-presenting cell (APC), e.g., a dendritic cell, phagocytoses exogenous antigens (bacteria or soluble antigens) and degrades them partially. Antigen fragments are presented by MHC class II molecules to a CD4-positive Th-cell; the MHC-antigen complex on the APC is recognized by the T-cell receptor (TCR) and CD4 molecules on the Th-cell. The APC-Th-cell interaction leads to activation of the Th-cell. The activated Th-cell produces cytokines, resulting in the activation of macrophages (Th1 help), B cells (Th2 help; panel *b*), or cytotoxic T cells (panel *c*). **(b)** Antibody production. The presence of antigen and Th2-type cytokines causes proliferation and differentiation of B cells. Only B cells specific for the antigen become activated. The B cells, now called plasma cells, produce and secrete large amounts of antibody. Some B cells differentiate into memory cells. **(c)** Activation of cytotoxic T lymphocytes (CTLs). CTLs recognize nonself antigens expressed by MHC class I molecules on the surface of virally infected cells or tumor cells. Cytolytic proteins are produced by the CTL upon interaction with the target cell.

Immune response	Immune product	Accessory factors	Infectious agents
<i>Humoral</i>	IgG	Complement, neutrophils	Bacteria and viruses
	IgA	Alternative complement pathway	Microorganisms causing respiratory and enteric infections
	IgM	Complement, macrophages	(Encapsulated) bacteria
	IgE	Mast cells	Parasites
<i>Cell mediated</i>	CTL	Cytolytic proteins	Viruses and mycobacteria
	T _{DTH}	Macrophages	Viruses, mycobacteria, treponema (syphilis), fungi

Table 22.3 ■ Important immune products protecting against infectious diseases.

system by APCs. This may have consequences for the design of a particular vaccine (see “[Vaccine Design in Relation with the Immune Response](#)”).

Antibodies are the typical representatives of humoral immunity. An antibody belongs to one of four different immunoglobulin classes (IgM, IgG, IgA, or IgE) (cf. Chap. 7). Upon immunization, B cells expressing specific antibodies on their cell surface (representing a fifth immunoglobulin class, IgD) bind intact antigen and are activated. The surface-bound antibodies bind specific epitopes of the pathogen, and in close cooperation with T-helper cells (T_h-cells), the B cell becomes activated eventually resulting in massive clonal proliferation. The proliferated B cells are called plasma cells and excrete large amounts of soluble antibodies (Fig. 22.4 panel b). Antibodies are able to prevent infection or disease by several mechanisms:

1. Binding of antibody covers the antigen with Fc (constant fragment), the “rear end” of immunoglobulins. Phagocytic cells, like macrophages, express surface receptors for Fc. This allows targeting of the opsonized (antibody-coated) antigen to these cells, followed by enhanced phagocytosis.
2. Immune complexes (i.e., antibodies bound to target antigens) can activate complement, a system of proteins which then becomes cytolytic to bacteria, enveloped viruses, or infected cells.
3. Phagocytic cells may express receptors for complement factors associated with immune complexes. Binding of these activated complement factors enhances phagocytosis.
4. Viruses can be neutralized by antibodies through binding at or near receptor binding sites on the virus surface. This may prevent binding to and entry into the host cell.

Antibodies are effective against certain but not all infectious microorganisms. They may have limited value when CMI is the major protective mechanism. Of the cell types that are known to exhibit cytotoxicity, two are antigen sensitized. Because of their specificity, they are of special importance with respect to vaccine design:

1. Cytotoxic T lymphocytes (CTLs) react with target cells and kill them by release of cytolytic proteins like perforin. Target cells express nonself antigens like viral proteins or tumor antigens, by which they are identified. CTL responses, as antibody responses, are highly specific.
2. T cells involved in delayed-type hypersensitivity (T_{DTH}) are able to kill target cells as CTLs do but also have helper (T_{h1}-type, see below) functions that enable them to activate macrophages.

Other less specific cells involved in cytotoxic immune responses are natural killer cells (NK cells). They play a role in antibody-dependent cellular cytotoxicity (ADCC). NK cells recognize opsonized (antibody-coated) cells with their Fc receptors.

Besides plasma cells and cytotoxic cells, in many cases, memory B and T cells develop. Memory B cells do not produce soluble antibody, but on repeated antigen contact, their response time to develop into antibody-excreting plasma cells is shorter compared to naïve B cells.

The occurrence of different types of immune response to vaccines is the result of differences in antigen processing of the vaccine by APCs and, as a result, in the activation of T_h-cells (Figs. 22.3 and 22.4). Major histocompatibility complex (MHC) molecules play an important role in the presentation of processed antigens to T cells. Most cells expose MHC class I molecules and some also MHC class II molecules on their surface.

APCs carrying class II molecules process soluble, exogenous (extracellular) proteins or more complicated structures such as microorganisms (see Fig. 22.4, panel a). After their endocytosis, the proteins are subject to limited proteolysis before they return as peptides to the surface of the APC in combination with the class II molecules for presentation to a T-cell receptor of CD4-positive T_h-cells. The T_h-cells provide type 2 help necessary for the effector function of B cells. This type 2 help is characterized by the lymphokine pattern produced: interleukin 4 (IL-4), IL-5, IL-6, IL-10, and IL-13. These lymphokines trigger B cells, which eventually results in the production of IgM and IgG antibodies.

Cells carrying MHC class I molecules process endogenous (intracellularly produced) antigens like viral and tumor antigens and present them in combination with class I molecules on the cell surface (see Fig. 22.4, panel c). The class I-antigen combination on the APC is recognized by the T-cell receptor of CD8-positive CTLs. Th-cells provide help for the CTLs. For the induction of CMI (Fig. 22.4, panels a, c), type 1 help is needed (production of IL-2 and IL-12, interferon- γ , and tumor necrosis factor). Th-cells are CD4 positive, regardless whether they have Th1 or Th2 functions. There is increasing evidence that the Th1/Th2 balance is an important immunological parameter since some diseases coincide with Th1 (autoimmunity)- or Th2 (allergy)-type responses. Other T-helper cell phenotypes have been identified. Some play a role in autoimmune disease (e.g., T_{H17} -cells) or suppression of the immune response (e.g., T_{reg} -cells).

■ Vaccine Design in Relation with the Immune Response

For the rational design of a new vaccine, understanding of the mechanisms of the protective immunity to the pathogen against which the vaccine is developed is crucial. For instance, to prevent tetanus a high blood titer of antibody against tetanus toxin is required; in mycobacterial diseases such as tuberculosis, a macrophage-activating CMI is most effective; in case of an influenza virus infection, CTLs probably play a significant role besides antibodies. Importantly, the immune effector mechanisms triggered by a vaccine and, hence, the success of immunization depend not only on the nature of the protective components but also on their presentation form, the presence of adjuvants, and the route of administration.

The presentation form of the vaccine is one of the determinants that influence the extent and type of immune response that will be evoked (Pashine et al. 2005; Pulendran and Ahmed 2006). DCs and other APCs play a pivotal role in how the antigenic determinants of a vaccine will be processed and presented to T cells in the peripheral lymphoid organs. Through various PRRs, DCs are more or less able to “sense” the type of pathogen that is encountered. This determines the set of co-stimulatory signals and proinflammatory cytokines that will be generated by APCs when presenting the antigen to Th-cells in the peripheral lymphoid organs. For instance, pathogens or vaccines containing lipoproteins or peptidoglycans will trigger DCs via TLR-2, which predominantly generates a T_{H2} response, whereas stimulation of DCs through TLR-3, TLR-4, TLR-5, or TLR-8 is known to yield robust T_{H1} responses. Therefore, vaccines should be formulated in such a way that the appropriate T_H response will be triggered. This can be done by presenting the antigen

in its native format, as is the case for the classical vaccines, or by adding adjuvants that stimulate the desired response (see below).

The response by B cells is dependent upon the nature of the antigen and two types of antigens can be distinguished:

1. Thymus-independent antigens include certain linear antigens that are not readily degraded in the body and have a repeating determinant, such as bacterial polysaccharides. They are able to stimulate B cells without the T_H -cell involvement. Thymus-independent antigens do not induce immunological memory.
2. Thymus-dependent antigens provoke little or no antibody response in T-cell-depleted animals. Proteins are the typical representatives of thymus-dependent antigens. A prerequisite for thymus dependency is that a physical linkage exists between the sites recognized by B cells and those by T_H -cells. When a thymus-independent antigen is coupled to a carrier protein containing T_H -epitopes, it becomes thymus dependent. As a result, these conjugates are able to induce memory.

When the antigen is a protein, the epitopes can be continuous or discontinuous. Continuous epitopes involve linear peptide sequences (usually consisting of up to ten amino acid residues) of the protein (see Fig. 22.5, panel a). Discontinuous epitopes comprise amino acid residues sometimes far apart in the primary sequence, which are brought together through the unique folding of the protein (see Fig. 22.5, panel b). Antibody recognition of B-cell epitopes, whether continuous or discontinuous, is usually dependent on the conformation (= three-dimensional structure). T-cell epitopes, on the other hand, are continuous peptide sequences, the conformation of which does not seem to play a role in T-cell recognition.

■ Route of Administration

The immunological response to a vaccine is dependent on the route of administration. Most current vaccines are administered intramuscularly or subcutaneously. Parenteral immunization usually induces systemic immunity. However, mucosal (e.g., oral, intranasal, or intravaginal) immunization may be preferred, because it may induce both mucosal and systemic immunity. Mucosal surfaces are the common entrance of many pathogens, and the induction of a mucosal secretory IgA response may prevent the attachment and entry of pathogens into the host. For example, antibodies against cholera need to be in the gut lumen to inhibit adherence to and colonization of the intestinal wall. Also, orally administered *Salmonella typhi* not only invades the mucosal lining of the gut but also infects cells of the phagocytic system throughout the body,

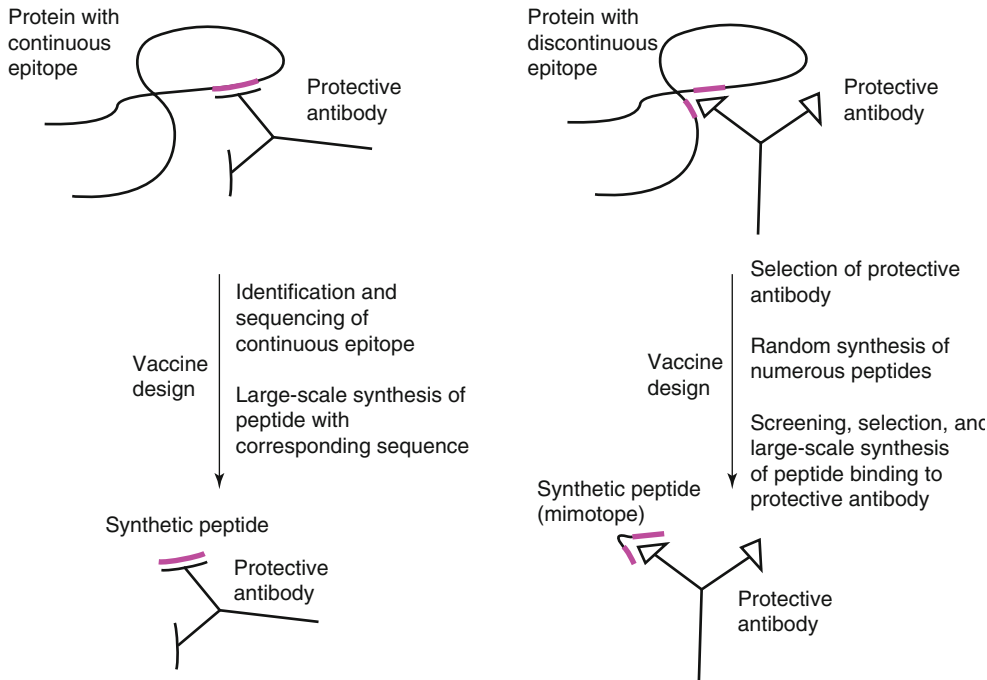


Figure 22.5 ■ Two approaches for the design of synthetic peptide vaccines. Panel *a*: identification and sequencing of a continuous epitope on an immunogenic protein is followed by the synthesis of peptides with the amino acid sequence corresponding to that of the epitope. Panel *b*: synthesis of peptides mimicking discontinuous epitopes that are determined by the three-dimensional structure of the immunogenic protein; a peptide that strongly binds to a protective antibody recognizing the discontinuous epitope is selected. The peptide (mimotope) does not necessarily contain the exact amino acid sequence of the constituent fragments that form the epitope.

thereby stimulating the production of both secretory and systemic antibodies, as well as CMI. Additional advantages of mucosal immunization are the ease of administration and the avoidance of systemic side effects (Holmgren and Czerkinsky 2005; Czerkinsky and Holmgren 2012). Up to now, however, successful mucosal immunization has only been achieved with a limited number of oral vaccines (e.g., oral polio, cholera, typhoid fever, and rotavirus vaccines) and a nasal influenza vaccine (FluMist). Most of these vaccines are based on attenuated (see later) versions of the pathogens for which the route of administration is the same as the natural route of infection.

Apart from mucosal routes, research groups are working on needle-free jet injection of powders and fluids and dermal delivery with microneedles (Kersten and Hirschberg 2004; Bal et al. 2010) (see Chap. 4). A prerequisite of these approaches is that they must be painless. In that case several immunizations can be given with monovalent vaccines, replacing one multivalent vaccine. Up to now, these products have not yet been registered.

CLASSICAL VACCINES

■ Classification

Classical vaccines originate from viruses or bacteria and can be divided in live attenuated vaccines and nonliving vaccines. In addition, three vaccine generations can be distinguished for nonliving vaccines. First-generation vaccines consist of an inactivated suspension

of the pathogenic microorganism. Little or no purification is applied. For second-generation vaccines, purification steps are applied, varying from the purification of a pathogenic microorganism (e.g., improved nonliving polio vaccine) to the complete purification of the protective component (e.g., polysaccharide vaccines). Third-generation vaccines are either a well-defined combination of protective components (e.g., acellular pertussis vaccine) or the protective component with the desired immunological properties (e.g., polysaccharides conjugated with a carrier protein). An overview of classical vaccines and their generations is given in Table 22.4.

■ Live Attenuated Vaccines

Before the introduction of recombinant DNA (rDNA) technology, a first step to improved live vaccines was the attenuation of virulent microorganisms by serial passage and selection of mutant strains with reduced virulence or toxicity. Examples are vaccine strains for oral polio vaccine, measles-mumps-rubella (MMR) combination vaccine, and tuberculosis vaccine consisting of bacille Calmette-Guérin (BCG). An alternative approach is chemical mutagenesis. For instance, by treating *Salmonella typhi* with nitrosoguanidine, a mutant strain lacking some enzymes that are responsible for the virulence was isolated (Germanier and Fuer 1975).

Live attenuated organisms have a number of advantages as vaccines over nonliving vaccines. After administration, live vaccines may replicate in the host

Type	Example	Marketed	Characteristics ^a
<i>Live</i>			
Viral	Adenovirus	Yes	Oral vaccine, US military services only
	Poliovirus (Sabin)	Yes	Oral vaccine
	Hepatitis A virus	No	
	Measles virus	Yes	
	Mumps virus	Yes	
	Rubella virus	Yes	
	Varicella zoster virus	Yes	
	Vaccinia virus	Yes	
	Yellow fever virus	Yes	
	Rotavirus	No	
	Influenza virus	No	
Bacterial	Bacille Calmette-Guérin	Yes	Whole organism
	Salmonella typhi	Yes	Whole organism, oral vaccine
<i>Nonliving (first-generation products)</i>			
Viral	Poliovirus (Salk)	Yes	Inactivated whole organisms
	Influenza virus	Yes	
	Japanese B encephalitis virus	Yes	
Bacterial	Bordetella pertussis	Yes	Purified, inactivated whole organisms
	Vibrio cholerae	Yes	
	Salmonella typhi	Yes	
<i>Nonliving (second-generation products)</i>			
Viral	Poliovirus	Yes	Subunit vaccine Plasma-derived hepatitis B surface antigen
	Rabies virus	Yes	
	Hepatitis A virus	Yes	
	Influenza virus	Yes	
	Hepatitis B virus	Yes	
Bacterial	Bordetella pertussis	Yes	Bacterial protein extract
	Haemophilus influenzae type b	Yes	Capsular polysaccharides
	Neisseria meningitidis	Yes	Capsular polysaccharides
	Streptococcus pneumoniae	Yes	Capsular polysaccharides
	Vibrio cholerae	Yes	Bacterial suspension + B subunit of cholera toxin
	Corynebacterium diphtheriae	Yes	Diphtheria toxoid
	Clostridium tetani	Yes	Tetanus toxoid
<i>Nonliving (third-generation products)</i>			
Viral	Measles virus	No	Subunit vaccine, ISCOM formulation
Bacterial	Bordetella pertussis	Yes	Mixture of purified protein antigens
	Haemophilus influenzae type b	Yes	Polysaccharide-protein conjugates
	Neisseria meningitidis	No	Polysaccharide-protein conjugates
	Streptococcus pneumoniae	No	Polysaccharide-protein conjugates

Source: Plotkin et al. (2008)

^aUnless mentioned otherwise, the vaccine is administered via the needle

Table 22.4 ■ Classical vaccines.

similar to their pathogenic counterparts. This confronts the host with a larger and more sustained dose of antigen, which means that few and low doses are required. In general, the vaccines give long-lasting humoral and cell-mediated immunity.

Live vaccines also have drawbacks. Live viral vaccines bear the risk that the nucleic acid sequence

is incorporated into the host's genome. Moreover, reversion to a virulent form may occur, although this is unlikely when the attenuated seed strain contains several mutations. Nevertheless, for diseases such as viral hepatitis, AIDS, and cancer, this drawback makes the use of classical live vaccines virtually unthinkable. Furthermore, it is important to recognize that

immunization of immune-deficient children with live organisms can lead to serious complications. For instance, a child with T-cell deficiency may become overwhelmed with BCG and die.

■ Nonliving Vaccines: Whole Organisms

An early approach for preparing vaccines is the inactivation of whole bacteria or viruses. A number of reagents (e.g., formaldehyde, glutaraldehyde) and heat are commonly used for inactivation. Examples of this first-generation approach are pertussis, cholera, typhoid fever, and inactivated polio vaccines. These nonliving vaccines have the disadvantage that little or no CMI is induced. Moreover, they more frequently cause adverse effects as compared to live attenuated vaccines and second- and third-generation nonliving vaccines.

■ Nonliving Vaccines: Subunit Vaccines

Diphtheria and Tetanus Toxoids

- Some bacteria such as *Corynebacterium diphtheriae* and *Clostridium tetani* form toxins. Antibody-mediated immunity to the toxins is the main protection mechanism against infections with these bacteria. Both toxins are proteins and are inactivated with formaldehyde for inclusion in vaccines. The immunogenicity of such toxoids is relatively low and was improved by adsorption of the toxoids to a suspension of aluminum salts. This combination of an antigen and an adjuvant is still used in combination vaccines.

Acellular Pertussis Vaccines

The relatively frequent occurrence of side effects of whole-cell pertussis vaccine was the main reason to develop subunit vaccines. The development of third-generation acellular pertussis vaccines in the 1980s exemplifies how a better insight into factors that are important for pathogenesis and immunogenicity can lead to an improved vaccine. It was conceived that a subunit vaccine consisting of a limited number of purified immunogenic components and devoid of (toxic) lipopolysaccharide would significantly reduce undesired effects. Four protein antigens important for protection have been identified. However, as yet there exists no consensus about the optimal composition of an acellular pertussis vaccine. Current vaccines contain different amounts of two to four of these proteins.

Polysaccharide Vaccines

Bacterial capsular polysaccharides consist of pathogen-specific multiple repeating carbohydrate epitopes, which are isolated from cultures of the pathogenic species. Plain capsular polysaccharides (second-generation

vaccines) are thymus-independent antigens that are poorly immunogenic in infants and show poor immunological memory when applied in older children and adults. The immunogenicity of polysaccharides is highly increased when they are chemically coupled to carrier proteins containing T_H-epitopes. This coupling makes them T cell dependent, which is due to the participation of T_H-cells that are activated during the response to the carrier. Examples of such third-generation polysaccharide conjugate vaccines include meningococcal type C, pneumococcal, and *Haemophilus influenzae* type b (Hib) polysaccharide vaccines that are included in many national immunization programs.

MODERN VACCINE TECHNOLOGIES

■ Modern Live Vaccines

Genetically Attenuated Microorganisms

Emerging insights in molecular pathogenesis of many infectious diseases make it possible to attenuate microorganisms very efficiently nowadays. By making multiple deletions, the risk of reversion to a virulent state during production or after administration can be virtually eliminated. A prerequisite for attenuation by genetic engineering is that the factors responsible for virulence and the life cycle of the pathogen are known in detail. It is also obvious that the protective antigens must be known: attenuation must not result in reduced immunogenicity.

An example of an improved live vaccine obtained by homologous genetic engineering is an experimental, oral cholera vaccine. An effective cholera vaccine should induce a local, humoral response in order to prevent colonization of the small intestine. Initial trials with *Vibrio cholerae* cholera toxin (CT) mutants caused mild diarrhea, which was thought to be caused by the expression of accessory toxins. A natural mutant was isolated that was negative for these toxins. Next, CT was detoxified by rDNA technology. The resulting vaccine strain, called CVD 103, is well tolerated by volunteers (Suharyono et al. 1992; Tacket et al. 1999) and challenge experiments with adult volunteers showed protection (Garcia et al. 2005).

Genetically attenuated live vaccines have the general drawbacks mentioned in the section about classically attenuated live vaccines. For these reasons, it is not surprising that homologous engineering is mainly restricted to pathogens that are used as starting materials for the production of subunit vaccines (see the section “[Subunit Vaccines](#),” below).

Live Vected Vaccines

A way to improve the safety or efficacy of vaccines is to use live, avirulent, or attenuated organisms as

Vector	Antigens from	Advantages of vector	Disadvantages of vector
<i>Viral</i>			
Vaccinia	RSV, HIV, VSV, rabies virus, HSV, influenza virus, EBV, Plasmodium spp. (malaria)	Widely used in man (safe) Large insertions possible (up to 41 kB)	Sometimes causing side effects Very immunogenic: repeated use difficult
Avipoxviruses (canarypox, fowlpox)	Rabies virus, measles virus	Abortive replication in man Low immunogenicity	
Poliovirus	Vibrio cholerae, influenza virus, HIV, chlamydia	Widely used in man (safe) Live/oral and inactivated/parenteral forms possible	Small genome
Adenoviruses	RSV, HBV, EBV, HIV, CMV	Oral route applicable	Small genome
Herpes viruses (HSV, CMV, varicella virus)	EBV, HBV	Large genome	
<i>Bacterial</i>			
Salmonella spp.	B. pertussis, HBV, Plasmodium spp., E. coli, influenza virus, streptococci, Vibrio cholerae, Shigella spp.	Strong mucosal responses	
Mycobacteria (BCG)	Borrelia burgdorferi (lyme disease)	Widely used in man (safe) Large insertions possible	
E. coli	Bordetella pertussis Shigella flexneri		

BCG bacille Calmette-Guérin, CMV cytomegalovirus, EBV Epstein-Barr virus, HBV hepatitis B virus, HIV human immunodeficiency virus, HSV herpes simplex virus, RSV respiratory syncytial virus, VSV vesicular stomatitis virus

Table 22.5 ■ Examples of recombinant live vaccines.

a carrier to express protective antigens from a pathogen. Both bacteria and viruses can be used for this purpose; some of them are listed in Table 22.5. Live vectored vaccines are created by recombinant technology, wherein one or more genes of the vector organism are replaced by one or more protective genes from the pathogen. Administration of such live vectored vaccines results in efficient and prolonged expression of the antigenic genes either by the vaccinated individual's own cells or by the vector organism itself (e.g., in case of bacteria as carriers).

Most experience has been acquired with vaccinia virus by using the principle that is schematically shown in Fig. 22.6. Advantages of vaccinia virus as vector include (i) its proven safety in humans as a smallpox vaccine, (ii) the possibility for multiple immunogen expression, (iii) the ease of production, (iv) its relative heat resistance, and (v) its various possible administration routes. A multitude of live recombinant vaccinia vaccines with viral and tumor antigens have been constructed, several of which have been tested in the clinic (Jaoko et al. 2008; Jacobs et al. 2009). It has been demonstrated that the products of genes coding for viral envelope proteins can be correctly processed and inserted into the plasma membrane of infected cells. Problems related with the side effects or immunogenicity of vaccinia virus may

be circumvented by the use of attenuated strains or poxviruses with a nonhuman natural host.

Adenoviruses can also be used as vaccine vectors (see also Chap. 24). Adenoviruses have several characteristics that make them suitable as vaccine vectors: (i) they can infect a broad range of both dividing and nondividing mammalian cells; (ii) transgene expression is generally high and can be further increased by using heterologous promoter sequences; (iii) adenovirus vectors are mostly replication deficient and do not integrate their genomes into the chromosomes of host cells, making these vectors very safe to use; and (iv) upon parenteral administration, adenovirus vectors induce strong immunity and evoke both humoral and cellular responses against the expressed antigen. A number of clinical trials with human adenovirus vectors (HAd5) expressing antigens of Ebola virus, human immunodeficiency virus (HIV), and severe acute respiratory syndrome (SARS) as vaccines against these diseases are currently in progress or have been terminated (Nayak and Herzog 2010). In a double-blind, phase II clinical trial to study the effectiveness of a HAd5-based vaccine against HIV-1 infection, 3,000 HIV-1 seronegative volunteers were either given the Ad5 vaccine or a placebo. Strikingly, there seemed to be an increased HIV-1 infection rate in the group that had received the Ad5 vaccine (Buchbinder et al. 2008).

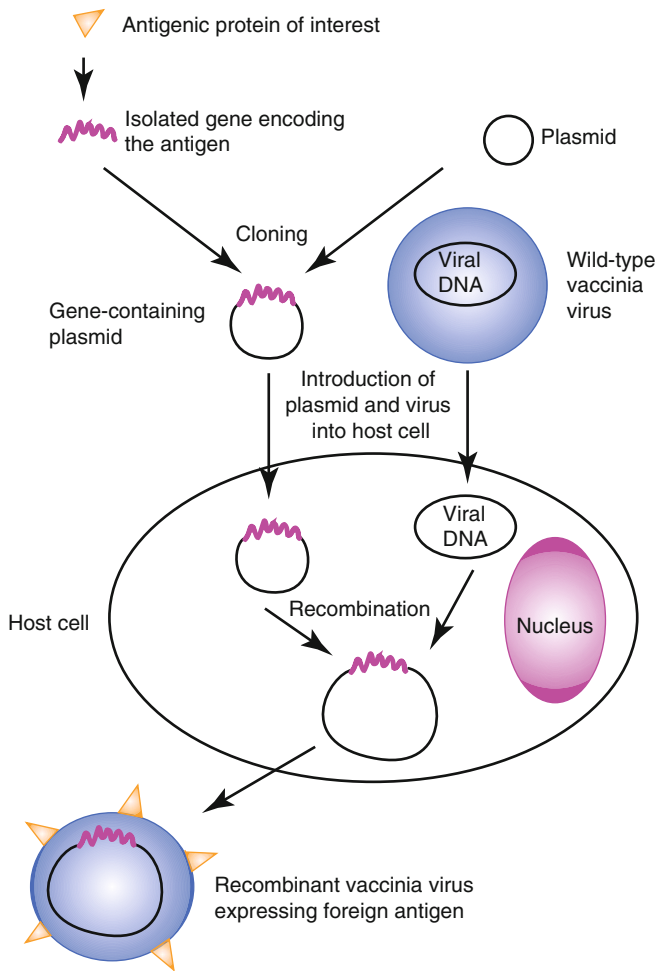


Figure 22.6 ■ Construction of recombinant vaccinia virus as a vector of foreign protein antigens. The gene of interest encoding an immunogenic protein is inserted into a plasmid. The plasmid containing the protein gene and wild-type vaccinia virus are then simultaneously introduced into a host cell line to undergo recombination of viral and plasmid DNA, after which the foreign protein is expressed by the recombinant virus.

A major limitation of the use of live vectored vaccines is the prevalence of preexisting immunity against the vector itself, which could neutralize the vaccine before the immune system can be primed. Such preexisting immunity has been described for adenoviral vectors, for which the prevalence of neutralizing antibodies can be as high as 90 % of the total population. The use of strains with no or low prevalence of preexisting immunity as live vectors is therefore recommended (Nayak and Herzog 2010; Ahi et al. 2011).

■ Modern Subunit Vaccines

Recombinant Protein Vaccines

To improve the yield, facilitate the production, and/or improve the safety of protein-based vaccines, protein antigens are nowadays often produced recombinantly,

i.e., expressed by host cells that are safe to handle and/or allow high expression levels.

Heterologous hosts used for the expression of immunogenic proteins include yeasts, bacteria, insect cells, plant cells, and mammalian cell lines. Hepatitis B surface antigen (HBsAg), which previously was obtained from plasma of infected individuals, has been expressed in bakers' yeast, *Saccharomyces cerevisiae* (Valenzuela et al. 1982; Vanlandschoot et al. 2002), and in mammalian cells, Chinese hamster ovary cells (Burnette et al. 1985; Raz et al. 2001), by transforming the host cell with a plasmid containing the HBsAg-encoding gene. Both expression systems yield 22-nm HBsAg particles (also called virus-like particles or VLPs) that are structurally identical to the native virus. Advantages are safety, consistent quality, and high yields. The yeast-derived vaccine has become available worldwide and appears to be as safe and efficacious as the classical plasma-derived vaccine.

The two human papillomavirus (HPV) vaccines currently on the market are produced as recombinant proteins which, like HBsAg, assemble spontaneously into virus-like particles. Antigens for Gardasil, a quadrivalent HPV vaccine, are produced in yeast, whereas antigens for the bivalent vaccine Cervarix are produced in insect cells.

Recombinant Peptide Vaccines

After identification of a protective epitope, it is possible to incorporate the corresponding peptide sequence through genetic fusion into a carrier protein, such as HBsAg, hepatitis B core antigen, and β -galactosidase (Francis and Larche 2005). The peptide-encoding DNA sequence is synthesized and inserted into the carrier protein gene. An example of the recombinant peptide approach is a malaria vaccine based on a 16-fold repeat of the Asn-Ala-Asn-Pro sequence of a *Plasmodium falciparum* surface antigen. The gene encoding this peptide was fused with the HBsAg gene, and the fusion product was expressed by yeast cells (Vreden et al. 1991). Genetic fusion of peptides with proteins offers the possibility to produce protective epitopes of toxic antigens derived from pathogenic species as part of nontoxic proteins expressed by harmless species. Furthermore, a uniform product is obtained in comparison with the variability of chemical conjugates (see the section "[Synthetic Peptide-Based Vaccines](#)," below).

Synthetic Peptide-Based Vaccines

In principle, a vaccine could consist of only the relevant epitopes instead of intact pathogens or proteins. Epitopes are small enough to be produced synthetically as peptides and a peptide-based vaccine would be much better defined than classical vaccines, making the concept of peptide vaccines attractive. However, it

turned out to be difficult to develop these vaccines, and today there are no licensed peptide-based vaccines available yet. Nevertheless, important progress has been made, and some synthetic peptide vaccines have now entered the clinic, e.g., for immunotherapy of cancer (Melief and van der Burg 2008). To understand the complexity of peptide vaccines, one has to distinguish the different types of epitopes.

Epitopes recognized by antibodies or B cells are very often conformation dependent (Van Regenmortel 2009). For this reason, it is difficult to identify them accurately. Manipulation of the antigen, such as digestion or the cloning of parts of the gene, will often affect B-cell epitope integrity. An accurate way of identifying epitopes is to elucidate the crystal structure of antigen-antibody complexes. This is difficult and time consuming, and although crystallography can reveal molecular interactions with unsurpassed detail, the molecular complex likely is much more dynamic in solution. Once the epitope is identified, synthesizing it as a functional peptide has proven to be difficult. The peptides need to be conformationally restrained. This can be achieved by cyclization of the peptide (Oomen et al. 2005) or by the use of scaffolds to synthesize complex peptide structures (Timmerman et al. 2009).

Regarding conformation, T-cell epitopes are less demanding because they are presented naturally as processed peptides by APCs to T cells. As a result, T-cell epitopes are linear. Here, we discern CD8 epitopes (8–10 amino acid residues; MHC class I restricted) and CD4 epitopes (>12 amino acid residues; MHC class II restricted). The main requirement is that they fit into binding grooves of MHC molecules with high enough affinity. Studies with peptide-based cancer vaccines have shown that these should contain both CD8 and CD4 epitopes in order to elicit a protective immune response. Furthermore, minimal peptides that can be externally loaded on MHC molecules of cells have been shown to induce less robust responses than longer peptides that require intracellular processing after uptake by DCs. Another point to consider is the variable repertoire of MHC molecules in a patient population, implying that a T-cell epitope-based peptide vaccine should contain several T-cell epitopes. Following these concepts, clinical trials with overlapping long peptide vaccines have shown promising results in the immunotherapy of patients with HPV-induced malignancies (Melief and van der Burg 2008).

Nucleic Acid Vaccines

Immunization with nucleic acid vaccines involves the administration of genetic material, plasmid DNA or messenger RNA, encoding the desired antigen. The encoded antigen is then expressed by the host cells and

after which an immune response against the expressed antigen is raised (Donnelly et al. 2005).

Plasmid DNA is produced by replication in *E. coli* or other bacterial cells and purification by established methods (e.g., density gradient centrifugation, ion-exchange chromatography). Up until now, plasmid DNA has been administered to animals and humans mostly via intramuscular injection. The favorable properties of muscle cells for DNA expression are probably due to their relatively low turnover rate, which prevents that plasmid DNA is rapidly dispersed in dividing cells. After intracellular uptake of the DNA, the encoded protein is expressed on the surface of host cells. After a single injection, the expression can last for more than 1 year. Besides intramuscular injection, subcutaneous, intradermal, and intranasal administrations also seem to be effective. Needleless injection into the skin of DNA-coated gold nanoparticles via a gene gun has been reported to require up to 1,000-fold less DNA when compared to intramuscular administration.

Nucleic acid vaccines offer the safety of subunit vaccines and the advantages of live recombinant vaccines. They can induce strong CTL responses against the encoded antigen. In addition, bacterial plasmids are also ideal for activating innate immunity as TLR-9 expressed on many phagocytic cells can recognize unmethylated bacterial DNA. The main disadvantage of nucleic acid immunization is the poor immunogenicity in man. Therefore, they often require, like subunit vaccines, adjuvants or delivery systems to boost the immune response against the DNA-encoded antigen(s). Nevertheless, DNA has proven to be very effective when used in combination with protein antigens in heterologous DNA-prime/protein-boost strategies. The long-term safety of nucleic acid vaccines remains to be established. The main pros and cons of nucleic acid vaccines are listed in Table 22.6. An advantage of RNA over DNA is that it is not able to incorporate into host DNA. A drawback of RNA, however, is that it is less stable than DNA. Nucleic acids coding for a variety of antigens have shown to induce protective, long-lived humoral and cellular immune responses in various species including man (Liu 2011). Examples of DNA vaccines that have been tested in clinical trials comprise plasmids encoding HIV-1 antigens and malaria antigens.

■ Reverse Vaccinology

Nowadays vaccines can be designed based on the information encoded by the genome of a particular pathogen (Masignani et al. 2002; Rappuoli and Covacci 2003). From many pathogens, the entire genomes have been sequenced and this number is growing (<http://cmr.tigr.org>). The genome sequence of a pathogen provides a complete picture of all proteins that can be

Advantages	Disadvantages
Low intrinsic immunogenicity of nucleic acids	Effects of long-term expression unknown
Induction of long-term immune responses	Formation of antinucleic acid antibodies possible
Induction of both humoral and cellular immune responses	Possible integration of the vaccine DNA into the host genome
Possibility of constructing multiple epitope plasmids	Concept restricted to peptide and protein antigens
Heat stability	Poor delivery
Ease of large-scale production	Poorly immunogenic in man

Table 22.6 ■ Advantages and disadvantages of nucleic acid vaccines.

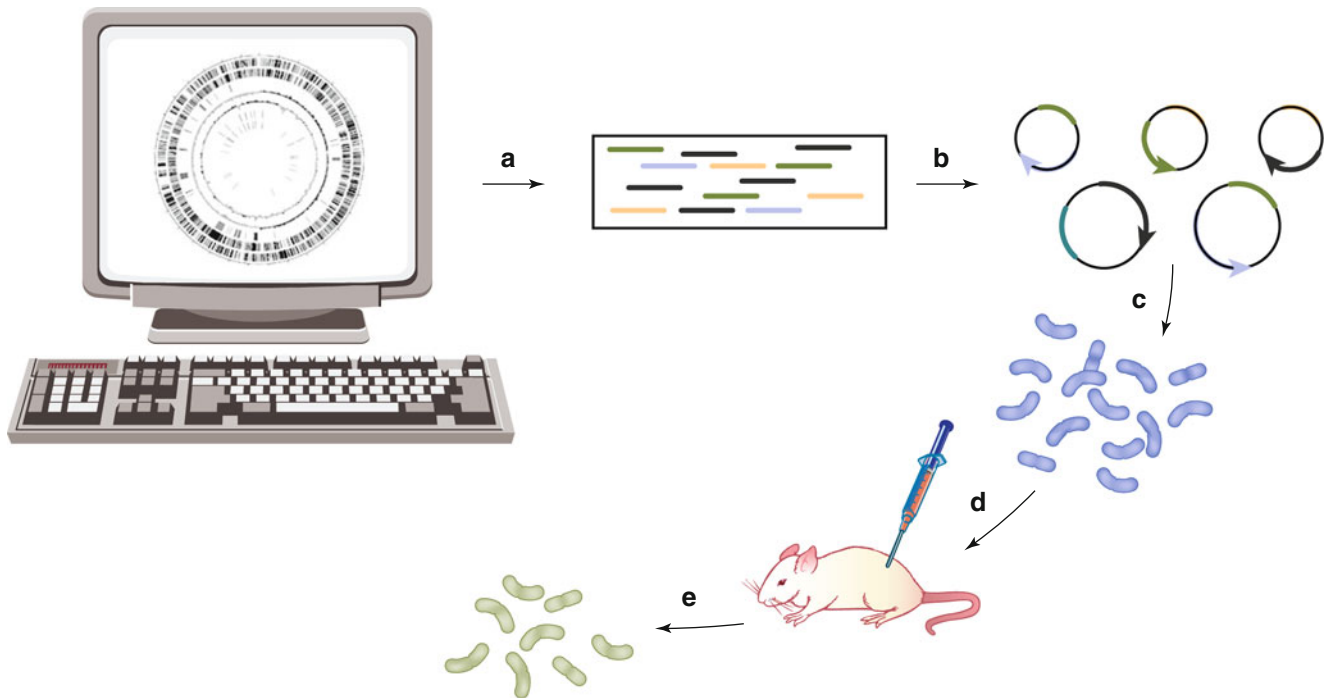


Figure 22.7 ■ Reverse vaccinology involves the analysis of genome sequences of pathogens in silico with the aim to identify potential antigens (a). These potential antigens can then be cloned (b), produced recombinantly (c), and subsequently used for immunological screening (d). The entire process leads to a quick identification of a limited number of vaccine candidates that can give protection against infection with the pathogen without the need to test all proteins produced by this pathogen (e) (Adapted from Scarselli et al. (2005)).

produced by the pathogens at any given time. Using computer algorithms, proteins that are either excreted or expressed on the surface of the pathogen, and thus most likely available for recognition by the host's immune system, can be identified. After recombinant production and purification, these vaccine candidates can be screened for immunogenicity in mice. From these, the best candidates can be selected and used as subunit vaccines (Fig. 22.7).

A big advantage of reverse vaccinology is the ease at which novel candidate antigens can be selected without the need to cultivate the pathogen. Furthermore, by

comparing genomes of different strains of a pathogen, conserved antigens can be identified that can serve as a "broad spectrum" vaccine, giving protection against all strains or serotypes of a given pathogen. One drawback of this approach is that it is limited to the identification of protein-based antigens.

Reverse vaccinology has been successfully used to identify novel antigens for a variety of pathogens, including *Neisseria meningitidis*, *Bacillus anthracis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Chlamydia pneumoniae*, and *Mycobacterium tuberculosis* (Sette and Rappuoli 2010).

■ Therapeutic Vaccines

Most classical vaccine applications are prophylactic: they prevent an infectious disease from developing. Besides prophylactic applications, vaccines may be used to treat already established diseases, such as infectious diseases, cancer, or drug addiction. Although the development of therapeutic vaccines is still in its infancy, some examples will be highlighted here.

Cancer Vaccines

Immunotherapy of cancer requires the activation of tumor-specific T cells, although humoral responses may in some cases (e.g., non-Hodgkin lymphoma) also be effective. Besides the synthetic long peptide-based approach already described above, there are several strategies to boost such a tumor-specific CTL response. Vaccines can be prepared from the patient's tumor itself by mixing irradiated tumor cells or cell extracts with bacterial adjuvants such as BCG to enhance their immunogenicity. Alternatively, heat shock proteins isolated from a patient's tumor that contain associated tumor antigens can be used as a tumor-specific vaccine. In combination with adjuvants, these heat shock proteins can be very potent in stimulating CTL responses against tumor cells as has been demonstrated in several clinical trials (Liu et al. 2002). Another approach is to genetically alter tumor cells in order to make them more immunogenic. Transfection of tumor cells with the gene encoding the co-stimulatory molecule B7 has resulted in direct activation of tumor-specific CTLs by the transformed tumor cells (Garnett et al. 2006). Similar results can be achieved by transforming tumor cells with gene encoding cytokines (e.g., GM-CSF, IL-2, IL-4, and IL-12).

Alternatively, the first step of the immune response can be optimized with an *in vitro* procedure. Dendritic cells are isolated from the patient and cultured *in vitro* in the presence of tumor antigen. The antigen loaded cells are subsequently given back to the patient. A licensed therapeutic prostate cancer vaccine is based on this principle (Cheever and Higano 2011). The potency of the vaccine is limited (a few months extension of life expectancy) and the costs are high (almost \$100,000 per patient), so there is substantial room for improvement.

Vaccines Against Drug Abuse

Therapeutic vaccines are also being developed for the treatment of drug abuse, such as addiction to nicotine, cocaine, or methamphetamine (Moreno and Janda 2009). The idea is to evoke a humoral immune reaction against the drug molecules. As most of these drugs have their addictive action within the central nervous system, antibodies raised against the drug molecules can prevent the passage of these molecules over the blood-brain barrier and thus prevent the addictive

effects. Many abused drugs are small nonprotein substances, which generally do not elicit an immune response as such. In order to activate the host immune system against these substances, they need to be conjugated to proteins, such as ovalbumin or diphtheria toxin. This approach has been effective in animal models. Late and mid-stage clinical studies, however, have shown disappointing results. A vaccine consisting of nicotine conjugated to virus-like particles failed in a phase 2 study, and a nicotine-protein conjugate vaccine failed in phase 3. This demonstrates the current lack of tools to predict or at least minimize the risk for late stage failures.

■ Systems Biology and Vaccines

One of the biggest problems in vaccine development is the inability to predict the efficacy and safety of new vaccines with other methods than phase 3 studies. Animal models are poorly predictive, and even immunological parameters in humans, like the induction of pathogen-neutralizing antibody responses, are not fully predictive. As a result, vaccine efficacy has to be measured in terms of reduction of disease. This is sometimes very difficult because symptoms of a disease can be caused by more than one pathogen (e.g., influenza-like illness). To measure protection, one has to detect the influenza virus in the group with influenza-like illness. In addition, to measure reduction of disease, the groups in the trial need to be very large because it is unknown who will get the disease. Sometimes tens of thousands of people are included in phase 3 trials.

Systems biology approaches that may limit these problems in the future are under development. The idea is to identify gene signatures that correlate with a protective immune response. This is done by a combination of gene expression analysis for, e.g., lymphocytes in the blood and functional assays like measurement of antibodies, cytokines, and cellular responses. Bioinformaticians try to unravel pathways and networks of genes involved in immune responses. Eventually, it may be possible to assign the activity of a limited number of genes to protective immune responses (Nakaya et al. 2011). Perhaps that such gene signatures can be used in future clinical trials (Pulendran et al. 2010). This approach would allow for reduction of the size of clinical studies, reduce the risk of late stage failure, and assess the significance of animal models in the preclinical phase of vaccine development.

PHARMACEUTICAL ASPECTS

■ Production

Except for synthetic peptides, the antigenic components of vaccines are derived from microorganisms or animal cells. For optimal expression of the required

vaccine component(s), these microorganisms or animal cells can be genetically modified. Animal cells are used for the cultivation of viruses and for the production of some subunit vaccine components and have the advantage that the vaccine components are released into the culture medium.

Three stages can be discerned in the manufacture of cell-derived vaccines: (1) cultivation or upstream processing, (2) purification or downstream processing, and (3) formulation. For the first two stages, the reader is referred to Chap. 3, whereas the formulation is addressed in the following section.

■ Formulation

Adjuvants, Immune Potentiators, and Delivery Systems

The success of immunization is not only dependent on the nature of the immunogenic components but also on their presentation form. Therefore, the search for effective and acceptable adjuvants is an important issue in modern vaccine development (Guy 2007). Adjuvants are defined as any material that can increase the humoral and cellular immune response against an antigen. Adjuvants can stimulate the immune system by several, not mutually exclusive mechanisms (Guy 2007): (i) a depot effect leading to slow antigen release and prolonged antigen presentation, (ii) attraction and stimulation of APCs by some local tissue damage and binding to PRRs present on APCs, and (iii) delivery of the antigen to regional lymph nodes by improved antigen uptake, transport, and presentation by APCs.

Colloidal aluminum salts (hydroxide, phosphate) are widely used in many classical vaccine formulations. A few other adjuvants, e.g., monophosphoryl lipid A in HPV vaccine and oil-in-water emulsions in influenza vaccines, have been introduced recently but most are in experimental testing or are used in veterinary vaccines. Table 22.7 shows a list of some well-known adjuvants.

Combination Vaccines

Since oral immunization is not possible for most available vaccines (see the section “Route of Administration” above), the strategy to mix individual vaccines in order to limit the number of injections has been common practice since many decades. Currently, vaccines are available containing up to six nonrelated antigens: diphtheria-tetanus-pertussis-hepatitis B-polio-Haemophilus influenzae type b vaccine. Another example is measles-mumps-rubella (MMR) vaccine, alone or in combination with varicella vaccine. Sometimes a vaccine contains antigens from several subtypes of a particular pathogen. Pneumococcal conjugate vaccine 13 (PVC13) is an example. This vaccine contains polysaccharides from thirteen pneumococcal strains, conjugated to a carrier protein to improve immunogenicity.

Adjuvant	Characteristics
Aluminum salts	Antigen adsorption is crucial
Lipid A and derivatives	Fragment of lipopolysaccharide, a bacterial endotoxin
MF59	Squalene-based oil-in-water emulsion
Muramyl peptides	Active fragments of bacterial cell walls
Saponins	Plant triterpene glycosides
NBP	Synthetic amphiphiles
DDA	Synthetic amphiphile
CpG	Non-methylated DNA sequences containing CpG-oligonucleotides
Cytokines	Interleukins (1, 2, 3, 6, 12), interferon- γ , tumor necrosis factor
Cholera toxin, B subunit	Mucosal adjuvant
Emulsions	Both water-in-oil and oil-in-water emulsions are used; often contain amphiphilic adjuvants
Liposomes	Phospholipid membrane vesicles; aqueous interior as well as lipid bilayer may contain antigens and/or adjuvants
ISCOMs	Micellar lipid-saponin complex; not suitable for soluble antigens
Microspheres	Biodegradable polymeric spheres, often poly(lactide-co-glycolide)

DDA dioctadecyldimethylammonium bromide, ISCOM immune stimulating complex, NBP nonionic block copolymers

Table 22.7 ■ Examples of adjuvants.

Combining vaccine components sometimes results in pharmaceutical as well as immunological problems. For instance, formaldehyde-containing components may chemically react with other components; an unstable antigen may need freeze drying, whereas other antigens should not be frozen. Components that are not compatible can be mixed prior to injection, if there is no short-term incompatibility. To this end, dual-chamber syringes have been developed.

From an immunological point of view, the immunization schedules of the individual components of combination vaccines should match. Even when this condition is met and the components are pharmaceutically compatible, the success of a combination vaccine is not warranted. Vaccine components in combination vaccines may exhibit a different behavior in vivo compared to separate administration of the components. For instance, enhancement (Paradiso et al. 1993) as well as suppression (Mallet et al. 2004) of humoral immune responses has been reported.

■ Characterization

Second- and third-generation classical vaccines and modern vaccines are better-defined products in terms of immunogenicity, structure, and purity. This means that the products can be characterized with a combination of appropriate biochemical, physicochemical, and immunochemical techniques (see Chap. 2). Vaccines have to meet similar standards as other biotechnological pharmaceuticals. The use of modern analytical techniques for the design and release of new vaccines is gaining importance. Currently, animal experiments are needed for quality control of many vaccines but *in vitro* analytical techniques may eventually (partly) substitute preclinical tests *in vivo*. During the development of the production process of a vaccine component, a combination of suitable assays can be defined. These assays can subsequently be applied during its routine production.

Column chromatographic (HPLC) and electrophoretic techniques like gel electrophoresis and capillary electrophoresis provide information about the purity, molecular weight, and electric charge of the vaccine component. Physicochemical assays comprise mass spectrometry and spectroscopy, including circular dichroism and fluorescence spectroscopy. Information is obtained mainly about the molecular weight and the conformation of the vaccine component. Immunochemical assays, such as enzyme-linked immunoassays and radioimmunoassays, are powerful methods for the quantification of the vaccine component. By using monoclonal antibodies (preferably with the same specificity as those of protective human antibodies) information can be obtained about the conformation and accessibility of the epitope to which the antibodies are directed. Moreover, the use of biosensors makes it possible to measure antigen-antibody interactions momentarily, allowing accurate determination of binding kinetics and affinity constants.

■ Storage

Depending on their specific characteristics, vaccines are stored as solution or as a freeze-dried formulation, usually at 2–8 °C. Their shelf life depends on the composition and physicochemical characteristics of the vaccine formulation and on the storage conditions and typically is in the order of several years. The quality of the container can influence the long-term stability of vaccines, e.g., through adsorption or pH changes resulting from contact with the vial wall. The use of pH indicators or temperature- or time-sensitive labels (“vial vaccine monitors,” which change color when exposed to extreme temperatures or after the expiration date) can avoid unintentional administration of inappropriately stored or expired vaccine.

CONCLUDING REMARKS

Despite the tremendous success of the classical vaccines, there are still many infectious diseases and other diseases (e.g., cancer) against which no effective vaccine exists. Although modern vaccines – like other biopharmaceuticals – are expensive, calculations may indicate cost-effectiveness for vaccination against many of these diseases. In addition, the growing resistance to the existing arsenal of antibiotics increases the need to develop vaccines against common bacterial infections. It is expected that novel vaccines against several of these diseases will become available, and in these cases, the preferred type of vaccine will be chosen from one of the different options described in this chapter.

SELF-ASSESSMENT QUESTIONS

■ Questions

1. What are the characteristics of the ideal vaccine? Which aspects should be addressed in the design of a vaccine in order to approach these characteristics?
2. How do antibodies neutralize antigens?
3. How do T cells discriminate between exogenous (extracellular) and endogenous (intracellular) antigens? What is the eventual result of these differences in responsiveness?
4. Which categories of classical vaccines exist and what are their characteristics?
5. Mention two main problems related with the immunogenicity of peptide-based vaccines. How are these problems dealt with?
6. Mention at least three advantages and three disadvantages of nucleic acid vaccines. Give one advantage and one disadvantage of RNA vaccines over DNA vaccines.
7. Which stages are discerned in the manufacture of cell-derived vaccines?
8. Mention two or more examples of currently available combination vaccines. Which pharmaceutical and immunological conditions have to be fulfilled when formulating combination vaccines?

■ Answers

1. The characteristics of the ideal vaccine are listed in Fig. 22.1.
2. Antibodies are able to neutralize antigens by at least four mechanisms:
 - (a) Fc-mediated phagocytosis
 - (b) Complement activation resulting in cytolytic activity
 - (c) Complement-mediated phagocytosis
 - (d) Competitive binding on sites that are crucial for the biological activity of the antigen
3. T cells are able to distinguish exogenous from endogenous antigens by the type of self-antigen

(MHC antigen) that is associated with processed antigen on the surface of the antigen-presenting cell. Processed antigen binds to MHC molecules, resulting in a cell surface located antigen/MHC complex. The complex is recognized by the T-cell receptor/CD4 or CD8 complex. A cell infected with a virus presents partially degraded viral antigen (i.e., endogenous antigen) complexed with class I MHC. The complex is recognized by CD8-positive T cells, resulting in the induction of cytotoxic T cells. Professional antigen-presenting cells like macrophages phagocytose exogenous antigen and present it in conjunction with class II MHC. CD4-positive T cells bind to the MHC-antigen complex. Subsequent B-cell or macrophage activation leads to antibody or inflammatory responses, respectively.

4. Classical vaccines consist of either live attenuated vaccines or nonliving vaccines. For nonliving vaccines, we discern three generations. The first generation comprises suspensions of inactivated, pathogenic organisms. Second-generation vaccines contain purified components, varying from whole organisms or extracts of organisms to purified single components. Third-generation vaccines are either well-defined mixtures of purified components or protective components formulated in an immunogenic presentation form. Examples of these categories are given in Table 22.4.
5. The first problem concerns the low immunogenicity of plain peptide vaccines. The immunogenicity can be improved by constructing multiple antigen peptides or by chemical coupling of peptides to carrier proteins. Alternatively, peptide epitopes can be incorporated into carrier proteins through genetic fusion of the peptide DNA with that of the carrier protein. The second problem of peptide antigens is that their conformation does not necessarily correspond to that of the epitope in the native protein, which in case of B-cell epitopes may lead to poor immune responses or responses to irrelevant peptide conformations. Solutions to this problem are sought in constraining the conformation of the synthetic peptide by chemical cyclization methods.
6. The advantages and disadvantages of nucleic acid vaccines are listed in Table 22.6. An advantage of RNA is that there is no risk of incorporation into host DNA. On the other hand, RNA is less stable than DNA.
7. The three production stages are (a) cultivation of cells and/or virus, (b) purification of the desired components, and (c) formulation of the vaccine.
8. Examples of combination vaccines include diphtheria-tetanus-pertussis(–polio) vaccines and measles-mumps-rubella(–varicella) vaccines. Prerequisites for combining vaccine components are:

- (a) Pharmaceutical compatibility of vaccine components and additives
- (b) Compatibility of immunization schedules
- (c) No interference between immune responses to individual components

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