

generalized immunosuppression is observed in malaria and African trypanosomiasis. This immune deficiency has been attributed to the production of immunosuppressive cytokines by activated macrophages and T cells and defects in T cell activation.

The consequences of parasitic infestations for health and economic development are devastating. Attempts to develop effective vaccines against these infections have been actively pursued for many years. Although the progress has been slow, elucidation of the fundamental mechanisms of immune responses to and immune evasion by parasites holds promise for the future.

Strategies for Vaccine Development

The birth of immunology as a science dates from Edward Jenner's successful vaccination against smallpox in 1796. The importance of prophylactic immunization against infectious diseases is best illustrated by the fact that worldwide programs of vaccination have led to the complete or nearly complete eradication of many of these diseases in many countries (see [Table 1.1](#)). The fundamental principle of vaccination is to administer a killed or attenuated form of an infectious agent, or a component of a microbe, that does not cause disease but elicits an immune response that provides protection against infection by the live, pathogenic microbe.

The success of vaccination in eradicating infectious disease depends on several properties of the microbes. Vaccines are most effective if the infectious agent does not establish latency, does not undergo antigenic variation, and does not interfere with the host immune response. It is difficult to effectively vaccinate against microbes such as HIV, which establishes latent infection, is highly variable, and inhibits host immunity. Vaccines are also most effective against infections that are limited to human hosts and do not have animal reservoirs.

Most vaccines in use today work by inducing humoral immunity. Antibodies are the only immune mechanism that prevents infections, by neutralizing and clearing microbes before they gain their foothold in the host. The best vaccines are those that stimulate the development of long-lived plasma cells that produce high-affinity antibodies and memory B cells. These aspects of humoral immune responses are best induced in the germinal center reaction (see [Chapter 12](#)), which requires help provided by protein antigen-specific CD4⁺ T follicular helper (Tfh) cells.

There are major challenges in developing effective vaccines against several important infections. The immunologic correlates of protection are often poorly defined. Fundamental questions about how to maximally stimulate durable memory, effective Tfh cells, and long-lived plasma cells remain unresolved. Clinical experience has taught us that the longevity of vaccine-induced protection varies greatly, being lifelong with hepatitis B antigen vaccines and quite short with many others. The reasons for this critical difference are unknown. It is hoped that continuing advances in basic immunology and in methods for analyzing immune responses in humans will lead to answers to these questions that will put vaccine development on a strong foundation of

mechanisms and basic understanding.

In the following section, we will summarize the approaches to vaccination that have been tried (Table 16.6) and their major value and limitations.

Attenuated and Inactivated Bacterial and Viral Vaccines

Some of the earliest (first generation) and most effective vaccines are composed of intact microbes that are treated in such a way that they are attenuated or killed so they can no longer cause disease while retaining their immunogenicity. The great advantage of attenuated microbial vaccines is that they elicit many of the innate and adaptive immune responses (both humoral and cell-mediated) that the pathogenic microbe would, and they are therefore the ideal way of inducing protective immunity. Live, attenuated bacteria were first shown by Louis Pasteur to confer specific immunity. The attenuated or killed bacterial vaccines currently in use generally induce limited protection and are effective for only short periods. Live, attenuated viral vaccines are usually more effective; polio, measles, and yellow fever are three good examples. The earliest approach for producing such attenuated viruses was repeated passage in cell culture. More recently, temperature-sensitive and gene deletion mutants have been generated to create attenuated viruses. Viral vaccines often induce long-lasting specific immunity, so immunization of children is sufficient for lifelong protection. The major concern with attenuated viral or bacterial vaccines is safety. The live-attenuated oral polio vaccine has nearly eradicated the disease, but in rare cases the virus in the vaccine is reactivated and itself causes paralytic polio. In fact, the success of worldwide vaccination is creating the problem that the vaccine-induced disease, although rare, could become more frequent than the naturally acquired disease. This potential problem may have to be tackled by reverting to the killed virus vaccine to complete the eradication program.

TABLE 16.6

Vaccine Approaches ^a

Type of Vaccine	Examples
Live attenuated or killed bacteria	Bacillus Calmette-Guérin, cholera
Live attenuated or killed viruses	Polio, influenza, rabies
Subunit (antigen) vaccines	Tetanus toxoid, diphtheria toxoid
Conjugate vaccines	<i>Haemophilus influenzae</i> , pneumococcus
Synthetic vaccines	Hepatitis (recombinant proteins)
Viral vectors	Clinical trials of human SARS-CoV-2 spike protein made by human

	and chimpanzee adenovirus vectors
DNA vaccines	Clinical trials ongoing for several infections
mRNA vaccines	Approved for COVID-19

^a The table lists selected examples of vaccines in use as of December 2020.

A widely used inactivated vaccine of considerable public health importance is the influenza vaccine. Influenza viruses grown in chicken eggs are used in two types of vaccines. The most common vaccine is a trivalent inactivated (killed) vaccine that is used in the flu shot that is given intramuscularly. Three of the most frequently encountered influenza strains are selected every year and incorporated in this vaccine. A second type of influenza vaccine involves the same three strains, but the vaccine is made up of live attenuated viruses and is used as a nasal spray. Two of the major limitations of current influenza vaccines is that they do not induce broadly neutralizing antibodies that recognize multiple strains of the virus and antibody-mediated protection is short lived.

Purified Antigen (Subunit) Vaccines

These second-generation vaccines were produced to eliminate the safety concerns associated with attenuated microbes. Subunit vaccines are composed of antigens purified from microbes or inactivated toxins and are usually administered with an adjuvant. One effective use of purified antigens as vaccines is for the prevention of diseases caused by bacterial toxins. Toxins can be rendered harmless without loss of immunogenicity, and such toxoids induce strong antibody responses. Diphtheria and tetanus are two infections whose life-threatening consequences have been largely controlled because of immunization of children with toxoid preparations. Vaccines composed of bacterial polysaccharide antigens are used against pneumococcus and *Haemophilus influenzae*. Because polysaccharides are T-independent antigens, they tend to elicit low-affinity antibody responses and are poorly immunogenic in infants (who do not mount strong T cell-independent antibody responses). High-affinity antibody responses may be generated against polysaccharide antigens even in infants by coupling the polysaccharides to proteins to form **conjugate vaccines** (Fig. 16.13). These vaccines elicit helper T cells to simulate germinal center reactions, which would not occur with simple polysaccharide vaccines. Such vaccines work like hapten-carrier conjugates and are a practical application of the principle of T and B cell cooperation (see Chapter 12). The currently used *H. influenzae*, pneumococcal, and meningococcal vaccines are conjugate vaccines. Purified protein vaccines stimulate helper T cells and antibody responses, but they do not generate potent CTLs. The reason for poor CTL development is that exogenous proteins (and peptides) usually enter the class II MHC pathway of antigen presentation (except in the special situation of cross-presentation). As a result, protein vaccines are not recognized efficiently by class I MHC-restricted CD8⁺ T cells.

Synthetic Antigen Vaccines

A goal of vaccine research has been to identify the most immunogenic microbial antigens or epitopes, to synthesize these in the laboratory, and to use the synthetic antigens as vaccines. It is possible to deduce the protein sequences of microbial antigens from nucleotide sequence data and to prepare large quantities of proteins by recombinant DNA technology. Vaccines made of recombinant DNA-derived antigens are now in use for hepatitis B virus and HPV. In the case of the most widely used HPV vaccine, which was developed to prevent cancers caused by the virus, recombinant viral proteins from four strains (HPV 6, 11, 16, and 18) are made in yeast and combined with an adjuvant. HPV 6 and 11 are common causes of warts, and HPV 16 and 18 are the HPV strains most often linked to cervical cancer.

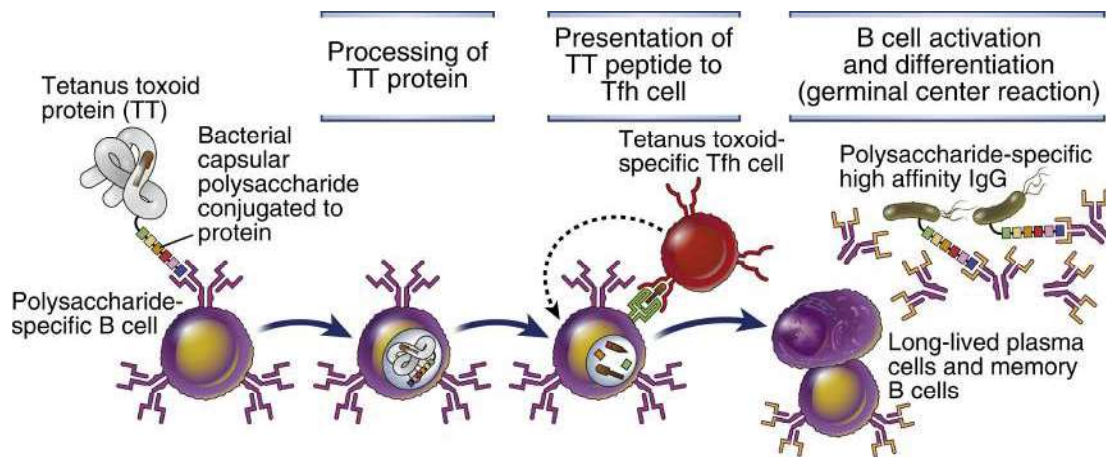


FIGURE 16.13 Conjugate vaccines. Conjugates of bacterial polysaccharides and a protein carrier (in this case, tetanus toxoid [TT]) induce potent high-affinity antibody responses because the protein carrier recruits helper T cells into the reaction. Note that the antibody produced is specific for the polysaccharide. *IgG*, Immunoglobulin G; *Tfh*, T follicular helper.

Live Viral Vaccines Involving Recombinant Viruses

Another approach for vaccine development is to introduce genes encoding microbial antigens into a noncytopathic virus and to infect individuals with this virus. Thus, the virus serves as a source of the antigen in an inoculated individual. The great advantage of viral vectors is that they, like other live viruses, induce the full complement of immune responses, including strong CTL responses. This technique has been used most commonly with vaccinia virus vectors, and more recently with canarypox viral vectors, which are not pathogenic in humans. Inoculation of such recombinant viruses into many species of animals induces both humoral and cell-mediated immunity against the antigen produced by the foreign gene (and, of course, against antigens of the viral

vectors as well). A potential problem with recombinant viruses is that the viruses may infect host cells, and even though they are not pathogenic, they may produce antigens that stimulate CTL responses that kill the infected host cells. Also, the nonpathogenic virus could recombine with host viruses or gene sequences and become virulent. These safety concerns have limited widespread use of viral vectors for vaccine delivery. One approach that overcomes many of these issues and concerns is the use of live recombinant hybrid vaccines that are non-replicating. An adenovirus 26 vector (humans generally lack antibodies to this adenovirus) and a chimpanzee adenovirus vector have been used to generate vaccines to a number of viruses, including Ebola virus, Zika virus, and SARS-CoV-2. Non-replicating adenoviruses infect numerous host cells and thus produce a significant amount of the viral antigen.

DNA Vaccines

An interesting method of vaccination was developed on the basis of an unexpected observation. Inoculation of a plasmid containing complementary DNA (cDNA) encoding a protein antigen leads to humoral and cell-mediated immune responses to the antigen. It is likely that APCs, such as DCs, are transfected by the plasmid and the cDNA is transcribed and translated into immunogenic protein that elicits specific responses. Bacterial plasmids are rich in unmethylated CpG nucleotides that are recognized by TLR9 in DCs and other cells, thereby eliciting an innate immune response that enhances adaptive immunity (see [Chapter 4](#)). Therefore, plasmid DNA vaccines could be effective even when administered without adjuvants. The ability to store DNA without refrigeration for use in the field also makes this technique promising. However, DNA vaccines have not been as effective as hoped in clinical trials, mainly because the first generation of these vaccines did not produce adequate amounts of the immunogen. Studies with newer vectors for DNA vaccination are currently in progress.

mRNA Vaccines

Another relatively recent mode of vaccination uses messenger RNA (mRNA) encoding microbial antigens. The main advantages of mRNA vaccines are the ease with which they can be rapidly developed, the ability to bypass the need for the large-scale manufacture and purification of protein antigens (thereby greatly reducing the cost), and the ability to combine mRNAs encoding many different protein antigens from a pathogen into a single vaccine. Although initial attempts to use mRNA were unsuccessful, largely because of stability issues, a number of recent advances have made mRNA vaccination a practical modality. One major advance is modifications of the mRNA itself. These modifications include addition of a synthetic 5' cap and a long poly A-tail to increase stability, altering 5' and 3' untranslated regions of the mRNA to enhance both translation and stability, and codon optimization of the coding portions to enhance translatability. Current mRNA vaccines for COVID-19 retain some ability to activate innate immunity by triggering RNA sensors. The mRNA is encapsulated in lipid nanoparticles that facilitate uptake by cells, including dendritic cells, and also

function as an adjuvant. Another approach to mRNA vaccines, not yet in clinical use, involves linking the mRNA to a modified alphavirus RNA genome that allows for self-replication, thus allowing many copies of the vaccine to be generated in recipient cells.

Adjuvants and Immunomodulators

The initiation of T cell–dependent immune responses against protein antigens requires that the antigens be administered with adjuvants. Most adjuvants elicit innate immune responses, with increased expression of costimulators and production of cytokines, such as IL-12, that stimulate T cell growth and differentiation. Heat-killed bacteria are powerful adjuvants that are commonly used in experimental animals. However, the severe local inflammation that such bacteria trigger precludes their use as adjuvants in humans. Much effort is currently being devoted to development of safe and effective adjuvants for clinical use. Only a few are approved for patients: aluminum hydroxide gel (which appears to promote mostly B cell responses); a bacterial product, monophosphoryl lipid A, alone or with aluminum salt; and a lipid formulation called squalene that may activate phagocytes. Recently, CG-rich oligonucleotides (CpG DNA) have been approved as an adjuvant for hepatitis B vaccines; by activating TLR9, these agents elicit potent innate immune reactions. An alternative to adjuvants is to administer natural substances that stimulate T cell responses together with antigens. As mentioned, plasmid DNA and some mRNA formulations have intrinsic adjuvant-like activities, and it is possible to incorporate costimulators (e.g., B7 molecules) or cytokines into plasmid DNA vaccines. These interesting ideas remain experimental.

Passive Immunization

Protective immunity also can be conferred by passive immunization, for instance, by transfer of specific antibodies. In the clinical situation, passive immunization is most commonly used for rapid treatment of potentially fatal diseases caused by toxins, such as tetanus, and for protection from rabies, hepatitis, and SARS-CoV-2. Antibodies against snake venom can be lifesaving when administered after poisonous snakebites. Convalescent plasma has been used in cases of Ebola and COVID-19. Recombinant monoclonal neutralizing antibodies are now utilized as a therapy for COVID-19. Passive immunity, using current approaches, is short-lived because the host does not respond to the immunization, and protection lasts only as long as the injected antibody persists. Moreover, passive immunization does not induce memory, so an immunized individual is not protected against subsequent exposure to the toxin or microbe. However, based on the successful identification of human broadly neutralizing monoclonal antibodies against pathogens, such as HIV and the flu virus, newer attempts for long-term passive immunization using a process called vectored immunoprophylaxis have been developed. In this approach, adeno-associated viral vectors are used to introduce cloned human Ig heavy and light chain genes for a neutralizing antibody into human subjects. The goal is to have injected humans synthesize a specific protective broadly neutralizing antibody for an extended period. Clinical trials have been initiated.