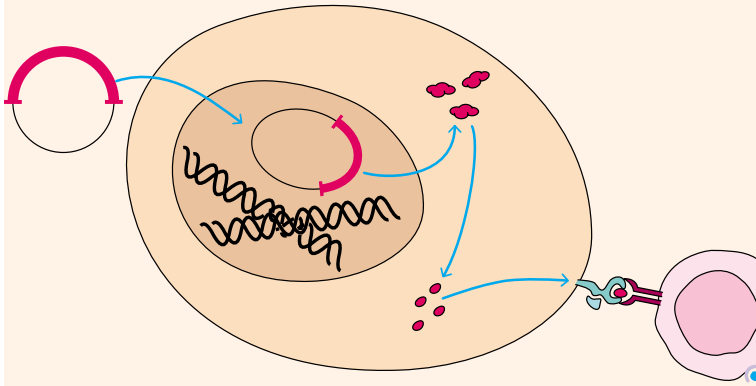
chapter 18

Vaccines



Vaccination with DNA

THE DISCIPLINE OF IMMUNOLOGY HAS ITS ROOTS IN the early vaccination trials of Edward Jenner and Louis Pasteur. Since those pioneering efforts, vaccines have been developed for many diseases that were once major afflictions of mankind. The incidence of diseases such as diphtheria, measles, mumps, pertussis (whooping cough), rubella (German measles), poliomyelitis, and tetanus has declined dramatically as vaccination has become more common. Clearly, vaccination is a cost-effective weapon for disease prevention. Perhaps in no other case have the benefits of vaccination been as dramatically evident as in the eradication of smallpox, one of mankind's long-standing and most terrible scourges. Since October 1977, not a single naturally acquired smallpox case has been reported anywhere in the world. Equally encouraging is the predicted eradication of polio. The last recorded case of naturally acquired polio in the Western Hemisphere occurred in Peru in 1991, and the World Health Organization (WHO) predicts that paralytic polio will be eradicated throughout the world within the next few years. A new addition to the weapons against childhood disease is a vaccine against bacterial pneumonia, a major cause of infant death.

A crying need remains for vaccines against other diseases. Every year, millions throughout the world die from malaria, tuberculosis, and AIDS, diseases for which there are no effective vaccines. It is estimated by the World Health Organization that 16,000 individuals a day, or 5.8 million a year, become infected with HIV-1, the virus that causes AIDS. An effective vaccine could have an immense impact on the control of this tragic spread of death and disaster. In addition to the challenges presented by diseases for which no vaccines exist, there remains the need to improve the safety and efficacy of present vaccines and to find ways to lower their cost and deliver them efficiently to all who need them, especially in developing countries of the world. The WHO estimates that millions of infant deaths in the world are due to diseases that could be prevented by existing vaccines (see Clinical Focus).

The road to successful development of a vaccine that can be approved for human use, manufactured at reasonable cost, and efficiently delivered to at-risk populations is costly, long, and tedious. Procedures for manufacture of materials that can be tested in humans and the ways they are tested in clinical trials are regulated closely. Even those candidate vaccines that survive initial scrutiny and are approved for use in human trials are not guaranteed to find their way into common usage. Experience has shown that not every vaccine candidate that was successful in laboratory and animal studies prevents disease in humans. Some potential vaccines cause unacceptable side effects, and some may even worsen the disease they were meant to prevent. Live virus vaccines pose a special threat to those with primary or acquired immunodeficiency (see Chapter 19). Stringent testing is an absolute necessity, because vaccines will be given to large numbers of well persons. Adverse side effects, even those that occur at very low frequency, must be balanced against the potential benefit of protection by the vaccine.

Vaccine development begins with basic research. Recent advances in immunology and molecular biology have led to effective new vaccines and to promising strategies for finding new vaccine candidates. Knowledge of the differences in epitopes recognized by T cells and B cells has enabled immunologists to begin to design vaccine candidates to maximize activation of both arms of the immune system. As differences in antigen-processing pathways became evident, scientists began to design vaccines and to use adjuvants that maximize antigen presentation with class I or class II MHC molecules.

CLINICAL FOCUS

Vaccination: Challenges in the U.S. and Developing Countries

Many previously common childhood diseases are seldom seen in the United States, a testament to the effectiveness of vaccination. A major barrier to similar success in the rest of the world is the difficulty of delivering vaccines to all children. However, even at home the U.S. is becoming a victim of its own success. Some parents who have never encountered diseases now nearly vanquished in the U.S. do not consider it important to have their infants vaccinated or they may be lax in adhering to recommended schedules of immunization. Others hold the uninformed belief that the risks associated with vaccination outweigh the risk of infection. This flawed reasoning is fueled by periodic allegations of linkage between vaccination and various disorders, such as the report circulating recently of a causal relationship between vaccination and autism, a condition of unknown etiology. Most such reports are based solely on the coincidental timing of vaccination and onset of disease, or on limited sampling and poor statistical analyses. So far, no alleged associations have withstood scrutiny that included large population samples and acceptable statistical methods.

While children in this country are protected against a variety of once-deadly diseases, this protection depends on continuation of our immunization programs. Dependency on herd immunity is dangerous for both the individual and society. Adverse reactions to vaccines must be examined thoroughly, of course, and if a vaccine causes unacceptable side reactions, the vaccination program must be reconsidered. At the same time, anecdotal reports of disease brought on by vaccines, and unsupported beliefs, such as the contention that vaccines weaken the immune system, must be countered by correct information from trusted sources. To retreat from our progress in immunization by noncompliance will return us to the age when measles, mumps, whooping cough, and polio were part of the risk of growing up.

Children in the developing world suffer from a problem different from those in the United States. Examination of infant deaths worldwide shows that existing vaccines could save the lives of millions of children. As seen in the table, there are safe, effective vaccines for five of the top ten killers of children. Although the list of diseases in the table includes HIV, TB, and malaria, for which no vaccines are available, administration of the vaccines that are recommended for infants in the United States could cut child mortality in the world by approximately half.

What barriers exist to the achievement of worldwide vaccination and complete eradication of many childhood diseases? The inability to achieve higher levels of

Genetic engineering techniques can be used to develop vaccines to maximize the immune response to selected epitopes and to simplify delivery of the vaccines. This chapter describes the vaccines now in use and describes vaccine strategies, including experimental designs that may lead to the vaccines of the future.

Active and Passive Immunization

Immunity to infectious microorganisms can be achieved by active or passive **immunization**. In each case, immunity can be acquired either by natural processes (usually by transfer from mother to fetus or by previous infection by the organism) or by artificial means such as injection of antibodies or vaccines (Table 18-1, on page 416). The agents used for inducing passive immunity include antibodies from humans or animals, whereas active immunization is achieved by inoculation with microbial pathogens that induce immunity but do not cause disease or with antigenic components from the pathogens. This section describes current usage of passive and active immunization techniques.

Passive Immunization Involves Transfer of Preformed Antibodies

Jenner and Pasteur are recognized as the pioneers of vaccination, or induction of active immunity, but similar recognition is due to Emil von Behring and Hidesaburo Kitasato for their contributions to passive immunity. These investigators were the first to show that immunity elicited in one animal can be transferred to another by injecting it with serum from the first (see Clinical Focus, Chapter 4).

Passive immunization, in which preformed antibodies are transferred to a recipient, occurs naturally by transfer of maternal antibodies across the placenta to the developing fetus. Maternal antibodies to diphtheria, tetanus, streptococci, rubeola, rubella, mumps, and poliovirus all afford passively acquired protection to the developing fetus. Maternal

vaccination even in the United States is an indication of the difficulty of the task. Even if we assume that suitable vaccines have been developed and that compliance is universal, the ability to produce and deliver the vaccines everywhere is a profound challenge. The World Health Organization (WHO) has stated that the ideal vaccine would have the following properties:

- Affordable worldwide
- Heat stable
- Effective after a single dose
- Applicable to a number of diseases
- Administered by a mucosal route
- Suitable for administration early in life

Few, if any, vaccines in common use today conform to all of these properties. However, the WHO goals can guide us in the pursuit of vaccines useful for worldwide application. They further aid us in setting priorities, especially for development of the vaccines needed most in developing countries. For example, an HIV/AIDS vaccine that meets the WHO criteria could have an immediate effect on the world AIDS epidemic, whereas one that does not will require further development before it reaches the populations most at risk.

Immunization saves millions of lives, and viable vaccines are increasingly available. The challenge to the biomedical research community is to develop better, safer, cheaper, easier-to-administer forms of these vaccines so that worldwide immunization becomes a reality.

Estimated annual deaths worldwide of children under 5 years of age, by pathogen

Pathogen	Deaths (millions)
*Pneumococcus**	***1.2***
Measles	**1.1**
Hemophilus (a–f, nst)	***0.9***
Rotavirus**	0.8
Malaria	0.7
HIV	0.5
RSV	0.5
Pertussis	**0.4**
Tetanus	**0.4**
Tuberculosis	0.1

*Pathogens shown in bold are those for which an effective vaccine exists.

** A licensed vaccine is being tested for possible side-effects.

SOURCE: Adapted from Shann and Steinhoff, 1999, *Lancet* **354** (Suppl II):7–11.

antibodies present in colostrum and milk also provide passive immunity to the infant.

Passive immunization can also be achieved by injecting a recipient with preformed antibodies. In the past, before vaccines and antibiotics became available, passive immunization provided a major defense against various infectious diseases. Despite the risks (see Chapter 16) incurred by injecting animal sera, usually horse serum, this was the only effective therapy for otherwise fatal diseases. Currently, there are several conditions that warrant the use of passive immunization. These include:

- Deficiency in synthesis of antibody as a result of congenital or acquired B-cell defects, alone or together with other immunodeficiencies.
- Exposure or likely exposure to a disease that will cause complications (e.g., a child with leukemia exposed to varicella or measles), or when time does not permit adequate protection by active immunization.
- Infection by pathogens whose effects may be ameliorated by antibody. For example, if individuals who have not received up-to-date active immunization against tetanus suffer a puncture wound, they are given an injection of horse antiserum to tetanus toxin. The preformed horse antibody neutralizes any tetanus toxin produced by *Clostridium tetani* in the wound.

Passive immunization is routinely administered to individuals exposed to botulism, tetanus, diphtheria, hepatitis, measles, and rabies (Table 18-2). Passively administered antiserum is also used to provide protection from poisonous snake and insect bites. Passive immunization can provide immediate protection to travelers or health-care workers who will soon be exposed to an infectious organism and lack active immunity to it. Because passive immunization does not activate the immune system, it generates no memory response and the protection provided is transient.

For certain diseases such as the acute respiratory failure in children caused by respiratory syncytial virus (RSV), passive

TABLE 18-1 Acquisition of passive and active immunity

Type	Acquired through
Passive immunity	Natural maternal antibody
	Immune globulin*
	Humanized monoclonal antibody
	Antitoxin†
Active immunity	Natural infection
	Vaccines‡
	Attenuated organisms
	Inactivated organisms
	Purified microbial macromolecules
	Cloned microbial antigens
	Expressed as recombinant protein
	As cloned DNA alone or in virus vectors
	Multivalent complexes
	Toxoid§

*An antibody-containing solution derived from human blood, obtained by cold ethanol fractionation of large pools of plasma; available in intramuscular and intravenous preparations.

†An antibody derived from the serum of animals that have been stimulated with specific antigens.

‡A suspension of attenuated live or killed microorganisms, or antigenic portions of them, presented to a potential host to induce immunity and prevent disease.

§A bacterial toxin that has been modified to be nontoxic but retains the capacity to stimulate the formation of antitoxin.

TABLE 18-2 Common agents used for passive immunization

Disease	Agent
Black widow spider bite	Horse antivenin
Botulism	Horse antitoxin
Diphtheria	Horse antitoxin
Hepatitis A and B	Pooled human immune gamma globulin
Measles	Pooled human immune gamma globulin
Rabies	Pooled human immune gamma globulin
Respiratory disease	Monoclonal anti-RSV*
Snake bite	Horse antivenin
Tetanus	Pooled human immune gamma globulin or horse antitoxin

*Respiratory syncytial virus

immunization is the best preventative currently available. A monoclonal antibody or a combination of two monoclonal antibodies may be administered to children at risk for RSV disease. These monoclonal antibodies are prepared in mice but have been "humanized" by splicing the constant regions of human IgG to the mouse variable regions (see Chapter 5). This modification prevents many of the complications that may follow a second injection of the complete mouse antibody, which is a highly immunogenic foreign protein.

Although passive immunization may be an effective treatment, it should be used with caution because certain risks are associated with the injection of preformed antibody. If the antibody was produced in another species, such as a horse, the recipient can mount a strong response to the isotypic determinants of the foreign antibody. This anti-isotype response can cause serious complications. Some individuals, for example, produce IgE antibody specific for determinants on the injected antibody. Immune complexes of this IgE bound to the passively administered antibody can mediate systemic mast cell degranulation, leading to systemic anaphylaxis. Other individuals produce IgG or IgM antibodies specific for the foreign antibody, which form complement-activating immune complexes. The deposition of these complexes in the tissues can lead to type III hypersensitive reactions. Even when human gamma globulin is administered passively, the recipient can generate an anti-allotype response to the human immunoglobulin, although its intensity is usually much less than that of an anti-isotype response.

Active Immunization Elicits Long-Term Protection

Whereas the aim of passive immunization is transient protection or alleviation of an existing condition, the goal of active immunization is to elicit protective immunity and immunologic memory. When active immunization is successful, a subsequent exposure to the pathogenic agent elicits a heightened immune response that successfully eliminates the pathogen or prevents disease mediated by its products. Active immunization can be achieved by natural infection with a microorganism, or it can be acquired artificially by administration of a **vaccine** (see Table 18-1). In active immunization, as the name implies, the immune system plays an active role—proliferation of antigen-reactive T and B cells results in the formation of memory cells. Active immunization with various types of vaccines has played an important

role in the reduction of deaths from infectious diseases, especially among children.

Vaccination of children is begun at about 2 months of age. The recommended program of childhood immunizations in this country, updated in 2002 by the American Academy of Pediatrics, is outlined in Table 18-3. The program includes the following vaccines:

- Hepatitis B vaccine
- Diphtheria-pertussis (acellular)-tetanus (DPaT) combined vaccine
- Inactivated (Salk) polio vaccine (IPV); the oral (Sabin) vaccine is no longer recommended for use in the United States
- Measles-mumps-rubella (MMR) combined vaccine
- *Haemophilus influenzae* (Hib) vaccine
- Varicella zoster (Var) vaccine for chickenpox
- Pneumococcal conjugate vaccine (PCV); a new addition to the list.

In addition, hepatitis A vaccine at 18 months and influenza vaccines after 6 months are recommended for infants in high-risk populations.

The introduction and spreading use of various vaccines for childhood immunization has led to a dramatic decrease in the incidence of common childhood diseases in the United States (Figure 18-1). The comparisons of disease incidence in 1999 to that reported in the peak years show dramatic drops and, in one case, complete elimination of the disease in the United States. As long as widespread, effective immunization programs are maintained, the incidence of these childhood diseases should remain low. However, the occurrence of side reactions to a vaccine may cause a drop in its use, which can lead to re-emergence of that disease. For example, the side effects from the pertussis attenuated bacterial vaccine included seizures, encephalitis, brain damage, and even death. Decreased usage of the vaccine led to an increase in the inci-

TABLE 18-3 Recommended childhood immunization schedule in the United States, 2002

	AGE								
Vaccine*	Birth	1 mo	2 mos	4 mos	6 mos	12 mos	15 mos	18 mos	4–6 yrs
Hepatitis B†	+	+							
			+			+			
Diphtheria, tetanus, pertussis‡			+	+	+		+		+
H. influenzae, type b			+	+	+	+			
Inactivated polio§			+	+	+				+
Pneumococcal conjugate			+	+	+	+			
Measles, mumps, rubella						+			+
Varicella#						+			

*This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines. Bars indicate ranges of recommended ages. Any dose not given at the recommended age should be given as a "catch-up" immunization at any subsequent visit when indicated and feasible.

†Different schedules exist depending upon the HBsAg status of the mother. A first vaccination after the first month is recommended only if the mother is HBsAg negative.

‡DTaP (diphtheria and tetanus toxoids and acellular pertussis vaccine) is the preferred vaccine for all doses in the immunization series. Td (tetanus and diphtheria toxoids) is recommended at 11–12 years of age if at least 5 years have elapsed since the last dose.

§Only inactivated poliovirus (IPV) vaccine is now recommended for use in the United States. However, OPV remains the vaccine of choice for mass immunization campaigns to control outbreaks due to wild poliovirus.

#Varicella (Var) vaccine is recommended at any visit on or after the first birthday for susceptible children, i.e., those who lack a reliable history of chickenpox (as judged by a health-care provider) and who have not been immunized. Susceptible persons 13 years of age or older should receive 2 doses, given at least 4 weeks apart.

SOURCE: Adapted from the ECBT Web site (see references); approved by the American Academy of Pediatrics.

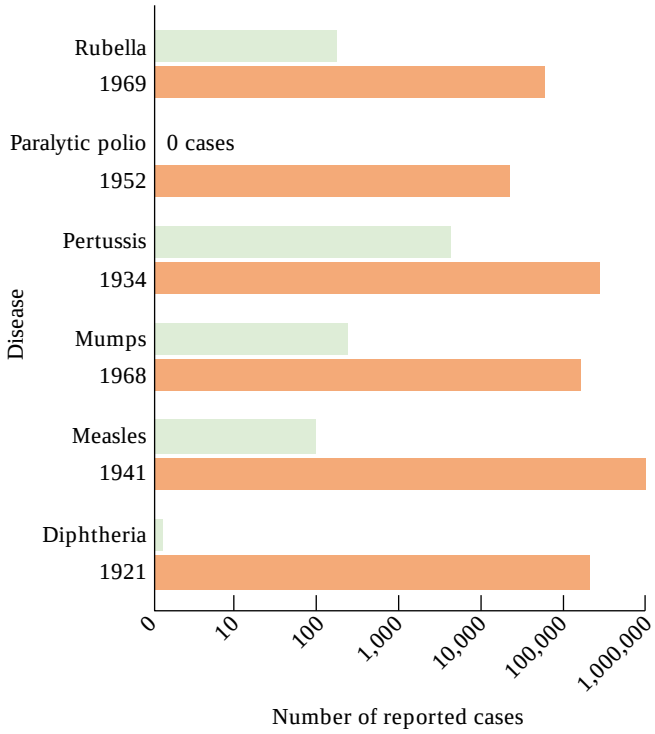


FIGURE 18-1 Reported annual number of cases of rubella (German measles), polio, pertussis (whooping cough), mumps, measles, and diphtheria in the United States in the peak year for which data are available (orange) compared with the number of cases of each disease in 1999 (green). Currently, vaccines are available for each of these diseases, and vaccination is recommended for all children in the United States. *[Data from Centers for Disease Control.]*

dence of whooping cough, with 7405 cases in 1998. The recent development of an acellular pertussis vaccine that is as effective as the older vaccine, but with none of the side effects, is expected to reverse this trend.

As indicated in Table 18-3, children typically require multiple boosters (repeated inoculations) at appropriately timed intervals to achieve effective immunity. In the first months of life, the reason for this may be persistence of circulating maternal antibodies in the young infant. For example, passively acquired maternal antibodies bind to epitopes on the DPT vaccine and block adequate activation of the immune system; therefore, this vaccine must be given several times after the maternal antibody has been cleared from an infant's circulation in order to achieve adequate immunity. Passively acquired maternal antibody also interferes with the effectiveness of the measles vaccine; for this reason, the MMR vaccine is not given before 12–15 months of age. In Third World countries, however, the measles vaccine is administered at 9 months, even though maternal antibodies are still present, because 30%–50% of young children in these countries contract the disease before 15 months of age. Multiple immunizations with the polio vaccine are required to ensure that an adequate immune response is generated to each of the three strains of poliovirus that make up the vaccine.

Recommendations for vaccination of adults depend on the risk group. Vaccines for meningitis, pneumonia, and influenza are often given to groups living in close quarters (e.g., military recruits) or to individuals with reduced immunity (e.g., the elderly). Depending on their destination, international travelers are also routinely immunized against such endemic diseases as cholera, yellow fever, plague, typhoid, hepatitis, meningitis, typhus, and polio. Immunization against the deadly disease anthrax had been reserved for workers coming into close contact with infected animals or products from them. Recently, however, suspected use of anthrax spores by terrorists or in biological warfare has widened use of the vaccine to military personnel and civilians in areas at risk of attack with this deadly agent.

Vaccination is not 100% effective. With any vaccine, a small percentage of recipients will respond poorly and therefore will not be adequately protected. This is not a serious problem if the majority of the population is immune to an infectious agent. In this case, the chance of a susceptible individual contacting an infected individual is so low that the susceptible one is not likely to become infected. This phenomenon is known as *herd immunity*. The appearance of measles epidemics among college students and unvaccinated preschool-age children in the United States during the mid- to late 1980s resulted partly from an overall decrease in vaccinations, which had lowered the herd immunity of the population (Figure 18-2). Among preschool-age children, 88% of those who developed measles were unvaccinated. Most of the college students who contracted measles had been vaccinated as children, but only once; the failure of the single vaccination to protect them may have resulted from the presence of passively acquired maternal antibodies that reduced their overall response to the vaccine. The increase in the incidence of measles prompted the recommendation that children receive two immunizations with the combined measles-mumps-rubella vaccine, one at 12–15 months of age and the second at 4–6 years.

The Centers for Disease Control (CDC) has called attention to the decline in vaccination rates and herd immunity among American children. For example, a 1995 publication reported that in California nearly one-third of all infants are unvaccinated and about half of all children under the age of 2 are behind schedule on their vaccinations. Such a decrease in herd immunity portends serious consequences, as illustrated by recent events in the newly independent states of the former Soviet Union. By the mid-1990s, a diphtheria epidemic was raging in many regions of these new countries, linked to a decrease in herd immunity resulting from decreased vaccination rates after the breakup of the Soviet Union. This epidemic, which led to over 157,000 cases of diptheria and 5000 deaths, is now controlled by mass immunization programs.

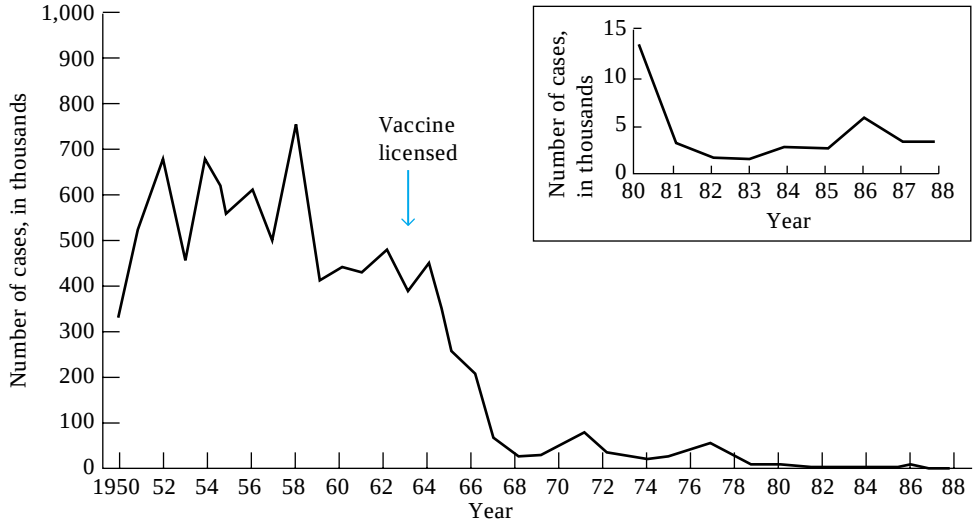


FIGURE 18-2 Introduction of the measles vaccine in 1962 led to a dramatic decrease in the annual incidence of this disease in the United States. Occasional outbreaks of measles in the 1980s (*inset*) occurred mainly among unvaccinated young children and among college students; most of the latter had been vaccinated, but only once, when they were young. *[Data from Centers for Disease Control.]*

Designing Vaccines for Active Immunization

Several factors must be kept in mind in developing a successful vaccine. First and foremost, the development of an immune response does not necessarily mean that a state of protective immunity has been achieved. What is often critical is which branch of the immune system is activated, and therefore vaccine designers must recognize the important differences between activation of the humoral and the cell-mediated branches. A second factor is the development of immunologic memory. For example, a vaccine that induces a protective primary response may fail to induce the formation of memory cells, leaving the host unprotected after the primary response to the vaccine subsides.

The role of memory cells in immunity depends, in part, on the incubation period of the pathogen. In the case of influenza virus, which has a very short incubation period (1 or 2 days), disease symptoms are already under way by the time memory cells are activated. Effective protection against influenza therefore depends on maintaining high levels of neutralizing antibody by repeated immunizations; those at highest risk are immunized each year. For pathogens with a longer incubation period, maintaining detectable neutralizing antibody at the time of infection is not necessary. The poliovirus, for example, requires more than 3 days to begin to infect the central nervous system. An incubation period of this length gives the memory B cells time to respond by producing high levels of serum antibody. Thus, the vaccine for polio is designed to induce high levels of immunologic memory. After immunization with the Salk vaccine, serum antibody levels peak within 2 weeks and then decline, but the memory response continues to climb, reaching maximal levels at 6 months and persisting for years (Figure 18-3). If an immunized individual is later exposed to the poliovirus, these memory cells will respond by differentiating into plasma cells that produce high levels of serum antibody, which defend the individual from the infection.

In the remainder of this chapter, various approaches to the design of vaccines—both currently used vaccines and experimental ones—are described, with an examination of their ability to induce humoral and cell-mediated immunity and the production of memory cells.

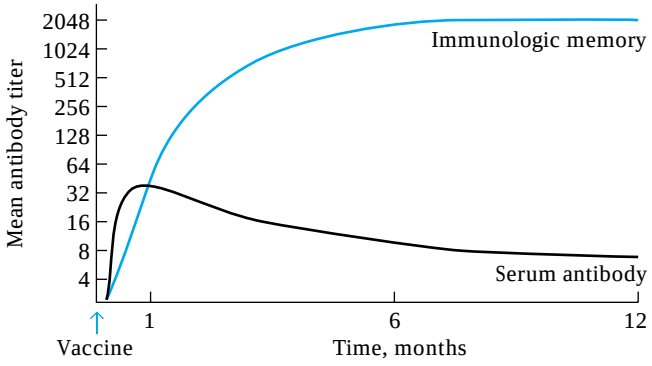


FIGURE 18-3 Immunization with a single dose of the Salk polio vaccine induces a rapid increase in serum antibody levels, which peak by 2 weeks and then decline. Induction of immunologic memory follows a slower time course, reaching maximal levels 6 months after vaccination. The persistence of the memory response for years after primary vaccination is responsible for immunity to poliomyelitis. *[From M. Zanetti et al., 1987,* Immunol. Today ***8****:18.]*

Whole-Organism Vaccines

As Table 18-4 indicates, many of the common vaccines currently in use consist of inactivated (killed) or live but attenuated (avirulent) bacterial cells or viral particles. The primary characteristics of these two types of vaccines are compared in Table 18-5 to one another and to DNA vaccines that are currently being tested for use in humans.

Attenuated Viruses and Bacteria Cause Immunity Without Disease

In some cases, microorganisms can be attenuated so that they lose their ability to cause significant disease (pathogenicity) but retain their capacity for transient growth within an inoculated host. Attenuation often can be achieved by growing a pathogenic bacterium or virus for prolonged periods under abnormal culture conditions. This procedure selects mutants that are better suited to growth in the abnormal culture conditions and are therefore less capable of growth in the natural host. For example, an attenuated strain of *Mycobacterium bovis* called **Bacillus Calmette-Guerin (BCG)** was developed by growing *M. bovis* on a medium containing increasing concentrations of bile. After 13 years, this strain had adapted to growth in strong bile and had become sufficiently attenuated that it was suitable as a vaccine for tuberculosis. The Sabin polio vaccine and the measles vaccine both consist of attenuated viral strains. The poliovirus used in the Sabin vaccine was attenuated by growth in monkey kidney epithelial cells. The measles vaccine contains a strain of rubella virus that was grown in duck embryo cells and later in human cell lines.

Attenuated vaccines have advantages and disadvantages. Because of their capacity for transient growth, such vaccines provide prolonged immune-system exposure to the individual epitopes on the attenuated organisms, resulting in increased immunogenicity and production of memory cells. As a consequence, these vaccines often require only a single immunization, eliminating the need for repeated boosters. This property is a major advantage in Third World countries, where epidemiologic studies have shown that roughly 20% of individuals fail to return for each subsequent booster. The ability of many attenuated vaccines to replicate within host cells makes them particularly suitable for inducing a cell-mediated response.

The Sabin polio vaccine, consisting of three attenuated strains of poliovirus, is administered orally to children on a sugar cube or in sugar liquid. The attenuated viruses colonize the intestine and induce protective immunity to all three strains of virulent poliovirus. Sabin vaccine in the intestines induces production of secretory IgA, which serves as an important defense against naturally acquired poliovirus. The vaccine also induces IgM and IgG classes of antibody. Unlike most other attenuated vaccines, which require a single immunizing dose, the Sabin polio vaccine requires boosters,

TABLE 18-4 Classification of common vaccines for humans

Disease or pathogen	Type of vaccine
WHOLE ORGANISMS	
Bacterial cells	
Anthrax	Inactivated
Cholera	Inactivated
Pertussis*	Inactivated
Plague	Inactivated
Tuberculosis	Live attenuated BCG†
Typhoid	Live attenuated
Viral particles	
Hepatitis A	Inactivated
Influenza	Inactivated
Measles	Live attenuated
Mumps	Live attenuated
Polio (Sabin)	Live attenuated
Polio (Salk)	Inactivated
Rabies	Inactivated
Rotavirus	Live attenuated
Rubella	Inactivated
Varicella zoster (chickenpox)	Live attenuated
Yellow fever	Live attenuated
PURIFIED MACROMOLECULES	
Toxoids	
Diphtheria	Inactivated exotoxin
Tetanus	Inactivated exotoxin
Capsular polysaccharides	
Haemophilus influenzae type b	Polysaccharide + protein carrier
Neissera meningitidis	Polysaccharide
Streptococcus pneumoniae	23 distinct capsular polysaccharides
Surface antigen	
Hepatitis B	Recombinant surface antigen (HBsAg)

*There is an now also an acellular pertussis vaccine consisting of toxoids and inactivated bacteria components.

†Bacillus Calmette-Guerin (BCG) is an avirulent strain of *Mycobacterium bovis.*

TABLE 18-5 Comparison of attenuated (live), inactivated (killed), and DNA vaccines

Characteristic	Attenuated vaccine	Inactivated vaccine	DNA vaccine
Production	Selection for avirulent organisms: virulent pathogen is grown under adverse culture conditions or prolonged passage of a virulent human pathogen through different hosts	Virulent pathogen is inactivated by chemicals or irradiation with γ-rays	Easily manufactured and purified
Booster requirement	Generally requires only a single booster	Requires multiple boosters	Single injection may suffice
Relative stability	Less stable	More stable	Highly stable
Type of immunity induced	Humoral and cell-mediated	Mainly humoral	Humoral and cell-mediated
Reversion tendency	May revert to virulent form	Cannot revert to virulent form	Cannot revert

because the three strains of attenuated poliovirus in the vaccine interfere with each other's replication in the intestine. With the first immunization, one strain will predominate in its growth, inducing immunity to that strain. With the second immunization, the immunity generated by the previous immunization will limit the growth of the previously predominant strain in the vaccine, enabling one of the two remaining strains to predominate and induce immunity. Finally, with the third immunization, immunity to all three strains is achieved.

A major disadvantage of attenuated vaccines is the possibility that they will revert to a virulent form. The rate of reversion of the Sabin polio vaccine (OPV) leading to subsequent paralytic disease is about one case in 2.4 million doses of vaccine. This reversion implies that pathogenic forms of the virus are being passed by a few immunized individuals and can find their way into the water supply, especially in areas where sanitation standards are not rigorous or where waste water must be recycled. This possibility has led to the exclusive use of the inactivated polio vaccine in this country (see Table 18-3). The projected eradication of paralytic polio (Figure 18-4) will be impossible as long as OPV is used anywhere in the world. The alternative inactivated Salk vaccine should be substituted as the number of cases decrease, although there are problems in delivering this vaccine in developing countries.

Attenuated vaccines also may be associated with complications similar to those seen in the natural disease. A small percentage of recipients of the measles vaccine, for example, develop post-vaccine encephalitis or other complications. As shown in Table 18-6 (page 423), however, the risk of vaccine-related complications is much lower than risks from infection. An independent study showed that 75 million doses of measles vaccine were given between 1970 and 1993, with an incidence of 48 cases of vaccine-related encephalopathy. The low in-cidence of this side effect compared with the rate of encephalopathy associated with infection argues for the efficacy of the vaccine. A more convincing argument for vaccination is the high death rate associated with measles infection even in developed countries.

Genetic engineering techniques provide a way to attenuate a virus irreversibly by selectively removing genes that are necessary for virulence. This has been done with a herpesvirus vaccine for pigs, in which the thymidine kinase gene was removed. Because thymidine kinase is required for the virus to grow in certain types of cells (e.g., neurons), removal of this gene rendered the virus incapable of causing disease. It is possible that similar genetic engineering techniques could eliminate the risk of reversion of the attenuated polio vaccine. More recently, a vaccine against rotavirus, a major cause of infant diarrhea, was developed using genetic engineering techniques to modify an animal rotavirus to contain antigens present on the human viruses.

Pathogenic Organisms Are Inactivated by Heat or Chemical Treatment

Another common approach in vaccine production is inactivation of the pathogen by heat or by chemical means so that it is no longer capable of replication in the host. It is critically important to maintain the structure of epitopes on surface antigens during inactivation. Heat inactivation is generally unsatisfactory because it causes extensive denaturation of proteins; thus, any epitopes that depend on higher orders of protein structure are likely to be altered significantly. Chemical inactivation with formaldehyde or various alkylating agents has been successful. The Salk polio vaccine is produced by formaldehyde inactivation.

Attenuated vaccines generally require only one dose to induce long-lasting immunity. Killed vaccines, on the other hand, often require repeated boosters to maintain the immune status of the host. In addition, killed vaccines induce a predominantly humoral antibody response; they are less effective than attenuated vaccines in inducing

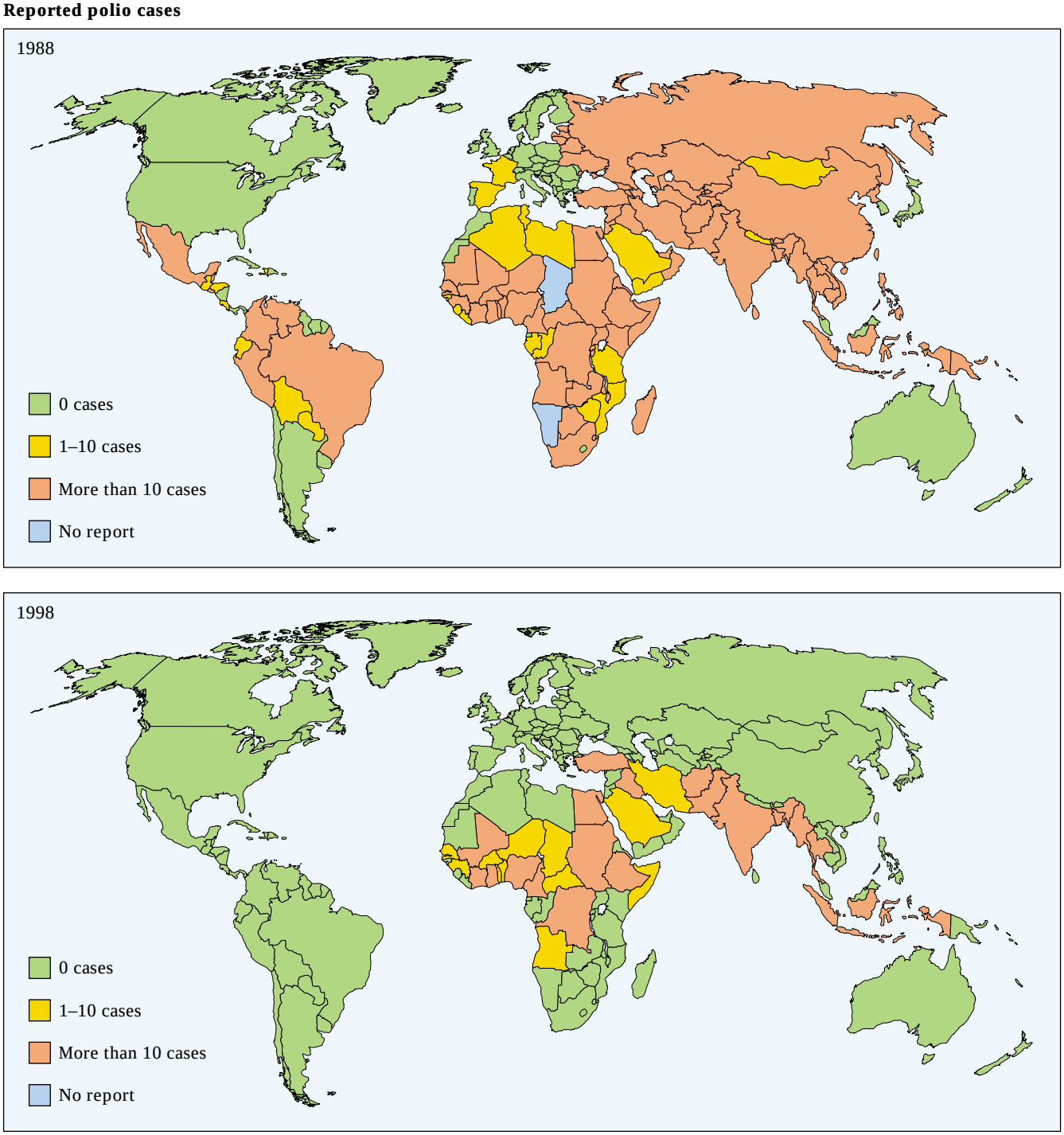


FIGURE 18-4 Progress toward the worldwide eradication of polio. Comparison of infection numbers for 1988 with those for 1998 show considerable progress in most parts of the world. Some experts question whether the use of live attenuated oral polio vaccine will cause reversion to pathogenic forms at a rate sufficiently high to prevent total eradication of this once prevalent crippling disease. *[Data from WHO.]*

cell-mediated immunity and in eliciting a secretory IgA response.

Even though they contain killed pathogens, inactivated whole-organism vaccines are still associated with certain risks. A serious complication with the first Salk vaccines arose when formaldehyde failed to kill all the virus in two vaccine lots, which caused paralytic polio in a high percentage of recipients.

TABLE 18-6 Risk of complications from natural measles infection compared with known risks of vaccination with a live attenuated virus in immunocompetent individuals

Complication	Risk after natural disease*	Risk after vaccination†
Otitis media	7–9%	0
Pneumonia	1–6%	0
Diarrhea	66%	0
Post-infectious encephalomyelitis	0.5–1 per 1000	1 per 1,000,000
SSPE	1 per 100,000	0
Thrombocytopenia	—‡	1 per 30,000§
Death	0.1–1 per 1000 (up to 5–15% in developing countries)	0

*Risk after natural measles are calculated in terms of events per number of cases.

†Risks after vaccination are calculated in terms of events per number of doses.

‡Although there have been several reports of thrombocytopenia occurring after measles including bleeding, the risk has not been properly quantified.

§This risk has been reported after MMR vaccination and cannot only be attributed to the measles component.

MMR = measles, mumps, and rubella.

SSPE = subacute sclerosing panencephalitis.

Purified Macromolecules as Vaccines

Some of the risks associated with attenuated or killed whole-organism vaccines can be avoided with vaccines that consist of specific, purified macromolecules derived from pathogens. Three general forms of such vaccines are in current use: inactivated exotoxins, capsular polysaccharides, and recombinant microbial antigens (see Table 18-4).

Bacterial Polysaccharide Capsules Are Used as Vaccines

The virulence of some pathogenic bacteria depends primarily on the antiphagocytic properties of their hydrophilic polysaccharide capsule. Coating of the capsule with antibodies and/or complement greatly increases the ability of macrophages and neutrophils to phagocytose such pathogens. These findings provide the rationale for vaccines consisting of purified capsular polysaccharides.

The current vaccine for *Streptococcus pneumoniae*, which causes pneumococcal pneumonia, consists of 23 antigenically different capsular polysaccharides. The vaccine induces formation of opsonizing antibodies and is now on the list of vaccines recommended for all infants. The vaccine for *Neisseria meningitidis*, a common cause of bacterial meningitis, also consists of purified capsular polysaccharides.

One limitation of polysaccharide vaccines is their inability to activate T_H cells. They activate B cells in a thymus-independent type 2 (TI-2) manner, resulting in IgM production but little class switching, no affinity maturation, and little, if any, development of memory cells. Several investigators have reported the induction of IgA-secreting plasma cells in humans receiving subcutaneous immunization with the pneumococcal polysaccharide vaccine. In this case, since T_H cells are not involved in the response, the vaccine may activate IgA-specific memory B cells previously generated by naturally-occurring bacterial antigens at mucosal surfaces. Because these bacteria have both polysaccharide and protein epitopes, they would activate T_H cells, which in turn could mediate class switching and memory-cell formation.

One way to involve T_H cells directly in the response to a polysaccharide antigen is to conjugate the antigen to some sort of protein carrier. For example, the vaccine for *Haemophilus influenzae* type b (Hib), the major cause of bacterial meningitis in children less than 5 years of age, consists of type b capsular polysaccharide covalently linked to a protein carrier, tetanus toxoid. The polysaccharide-protein conjugate is considerably more immunogenic than the polysaccharide alone, and because it activates T_H cells, it enables class switching from IgM to IgG. Although this type of vaccine can induce memory B cells, it cannot induce memory T cells specific for the pathogen. In the case of the Hib vaccine, it appears that the memory B cells can be activated to some degree in the absence of a population of memory T_H cells, thus accounting for the efficacy of this vaccine.

Toxoids Are Manufactured from Bacterial Toxins

Some bacterial pathogens, including those that cause diphtheria and tetanus, produce exotoxins. These exotoxins produce many of the disease symptoms that result from

infection. Diphtheria and tetanus vaccines, for example, can be made by purifying the bacterial exotoxin and then inactivating the toxin with formaldehyde to form a **toxoid**. Vaccination with the toxoid induces anti-toxoid antibodies, which are also capable of binding to the toxin and neutralizing its effects. Conditions for the production of toxoid vaccines must be closely controlled to achieve detoxification without excessive modification of the epitope structure. The problem of obtaining sufficient quantities of the purified toxins to prepare the vaccines has been overcome by cloning the exotoxin genes and then expressing them in easily grown host cells. In this way, large quantities of the exotoxin can be produced, purified, and subsequently inactivated.

Proteins from Pathogens Are Produced by Recombinant Techniques

Theoretically, the gene encoding any immunogenic protein can be cloned and expressed in bacterial, yeast, or mammalian cells using recombinant DNA technology. A number of genes encoding surface antigens from viral, bacterial, and protozoan pathogens have been successfully cloned into bacterial, yeast, insect, or mammalian expression systems, and the expressed antigens used for vaccine development. The first such recombinant antigen vaccine approved for human use is the hepatitis B vaccine. This vaccine was developed by cloning the gene for the major surface antigen of hepatitis B virus (HBsAg) and expressing it in yeast cells. The recombinant yeast cells are grown in large fermenters, and HBsAg accumulates in the cells. The yeast cells are harvested and disrupted by high pressure, releasing the recombinant HBsAg, which is then purified by conventional biochemical techniques. This recombinant hepatitis B vaccine has been shown to induce the production of protective antibodies. This vaccine holds much promise for the 250 million carriers of chronic hepatitis B worldwide!

Use of Synthetic Peptides as Vaccines Has Progressed Slowly

Although once considered very promising, the use of synthetic peptides as vaccines has not progressed as originally projected. Peptides are not as immunogenic as proteins, and it is difficult to elicit both humoral and cellular immunity to them. The use of conjugates and adjuvants can assist in raising protective immunity to peptides, but barriers to the widespread use of peptide vaccines remain and pose an interesting problem for immunologists. Most importantly, advances in techniques to produce recombinant proteins or fragments of proteins in transfected cell culture have removed the impetus to develop vaccines based on synthetic peptides. Nonetheless, there remains theoretical interest in immunity to them, and studies of peptide immunity may generate insights leading to new vaccines.

Construction of synthetic peptides for use as vaccines to induce either humoral or cell-mediated immunity requires an understanding of the nature of T-cell and B-cell epitopes. Ideally, vaccines for inducing humoral immunity should include peptides that form immunodominant B-cell epitopes. Such epitopes can be identified by determining the dominant antibody in the sera of individuals who are recovering from a disease and then testing various synthetic peptides for their ability to react with that antibody with high affinity. A successful vaccine must also generate a population of memory T_H cells; therefore the peptide should include immunodominant T-cell epitopes. Since MHC molecules differ in their ability to present peptides to T cells, MHC polymorphism within a species influences the level of T-cell response by different individuals to different peptides. Moreover, different subpopulations of T cells probably recognize different epitopes. Experiments by E. Sercarz have identified nonoverlapping amino acid sequences within hen egg-white lysozyme that induce a strong helper response to an antigen and other peptides that induce immunologic suppression. For example, immunization with the amino-terminal residues 1–17 of hen egg-white lysozyme suppressed the response to native lysozyme. By identifying suppressor peptides and eliminating them from synthetic vaccines, it might be possible to generate enhanced immunity.

Recombinant-Vector Vaccines

Genes that encode major antigens of especially virulent pathogens can be introduced into attenuated viruses or bacteria. The attenuated organism serves as a vector, replicating within the host and expressing the gene product of the pathogen. A number of organisms have been used for vector vaccines, including vaccinia virus, the canarypox virus, attenuated poliovirus, adenoviruses, attenuated strains of *Salmonella*, the BCG strain of *Mycobacterium bovis*, and certain strains of streptococcus that normally exist in the oral cavity.

Vaccinia virus, the attenuated vaccine used to eradicate smallpox, has been widely employed as a vector vaccine. This large, complex virus, with a genome of about 200 genes, can be engineered to carry several dozen foreign genes without impairing its capacity to infect host cells and replicate. The procedure for producing a vaccinia vector that carries a foreign gene from a pathogen is outlined in Figure 18-5. The genetically engineered vaccinia expresses high levels of the inserted gene product, which can then serve as a potent immunogen in an inoculated host. Like the smallpox vaccine, genetically engineered vaccinia vector vaccines can be administered simply by scratching the skin, causing a localized infection in host cells. If the foreign gene product expressed by the vaccinia is a viral envelope protein, it is inserted into the membrane of the infected host cell, inducing development of cell-mediated immunity as well as antibody-mediated immunity.

Other attenuated-vector vaccines may prove to be safer than the vaccinia vaccine. The canarypox virus has recently been tried as a vector vaccine. Like its relative vaccinia, the canarypox virus is large and easily engineered to carry multiple

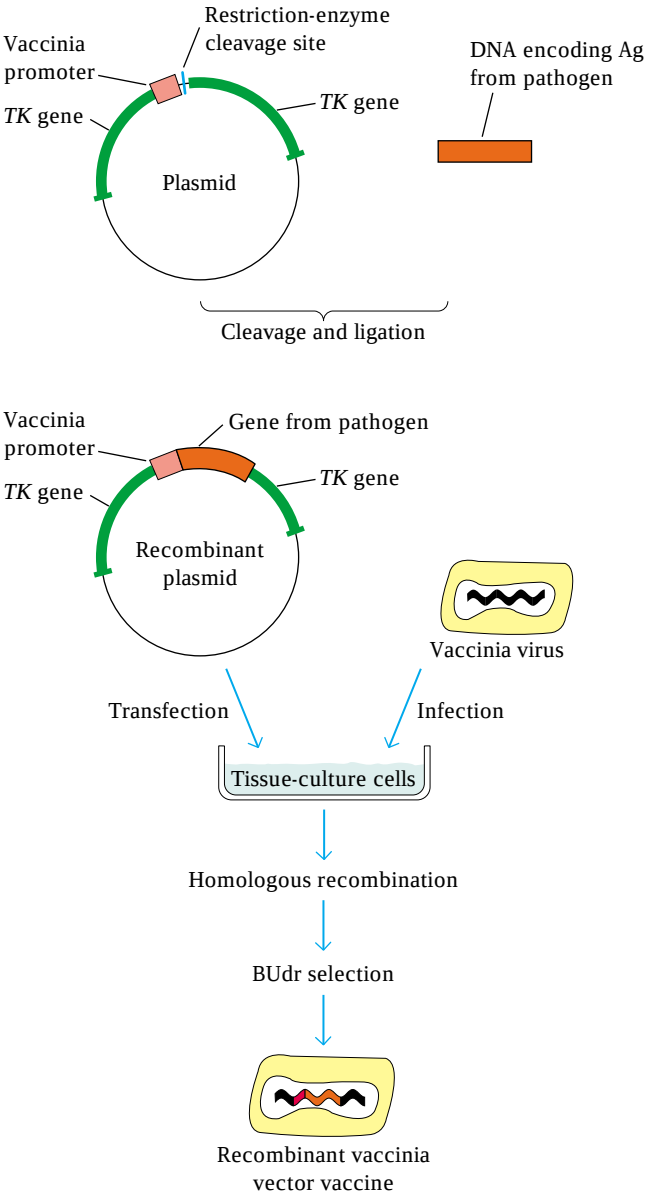


FIGURE 18-5 Production of vaccinia vector vaccine. The gene that encodes the desired antigen (orange) is inserted into a plasmid vector adjacent to a vaccinia promoter (pink) and flanked on either side by the vaccinia thymidine kinase (*TK*) gene (green). When tissue-culture cells are incubated simultaneously with vaccinia virus and the recombinant plasmid, the antigen gene and promoter are inserted into the vaccinia virus genome by homologous recombination at the site of the nonessential *TK* gene, resulting in a TK^- recombinant virus. Cells containing the recombinant vaccinia virus are selected by addition of bromodeoxyuridine (BUdr), which kills TK^+ cells. *[Adapted from B. Moss, 1985,* Immunol. Today ***6:****243.]*

genes. Unlike vaccinia, the canarypox virus does not appear to be virulent even in individuals with severe immune suppression. Another possible vector is an attenuated strain of *Salmonella typhimurium*, which has been engineered with genes from the bacterium that causes cholera. The advantage of this vector vaccine is that *Salmonella* infects cells of the mucosal lining of the gut and therefore will induce secretory IgA production. Effective immunity against a number of diseases, including cholera and gonorrhea, depends on increased production of secretory IgA at mucous membrane surfaces. Similar strategies using bacteria that are a normal part of oral flora are in development. The strategy would involve introduction of genes encoding antigens from pathogenic organisms into bacterial strains that inhabit the oral cavity or respiratory tract. Eliciting immunity at the mucosal surface could provide excellent protection at the portal used by the pathogen.

DNA Vaccines

In a recently developed vaccination strategy, plasmid DNA encoding antigenic proteins is injected directly into the muscle of the recipient. Muscle cells take up the DNA and the encoded protein antigen is expressed, leading to both a humoral antibody response and a cell-mediated response. What is most surprising about this finding is that the injected DNA is taken up and expressed by the muscle cells with much greater efficiency than in tissue culture. The DNA appears either to integrate into the chromosomal DNA or to be maintained for long periods in an episomal form. The viral antigen is expressed not only by the muscle cells but also by dendritic cells in the area that take up the plasmid DNA and express the viral antigen. The fact that muscle cells express low levels of class I MHC molecules and do not express costimulatory molecules suggests that local dendritic cells may be crucial to the development of antigenic responses to DNA vaccines (Figure 18-6).

DNA vaccines offer advantages over many of the existing vaccines. For example, the encoded protein is expressed in the host in its natural form—there is no denaturation or modification. The immune response is therefore directed to the antigen exactly as it is expressed by the pathogen. DNA vaccines also induce both humoral and cell-mediated immunity; to stimulate both arms of the immune response with non-DNA vaccines normally requires immunization with a live attenuated preparation, which introduces additional elements of risk. Finally, DNA vaccines cause prolonged expression of the antigen, which generates significant immunological memory.

The practical aspects of DNA vaccines are also very promising (Table 18-5). Refrigeration is not required for the handling and storage of the plasmid DNA, a feature that greatly lowers the cost and complexity of delivery. The same plasmid vector can be custom tailored to make a variety of proteins, so that the same manufacturing techniques can be used for different DNA vaccines, each encoding an antigen from a different pathogen. An improved method for administering these vaccines entails coating microscopic gold beads with the plasmid DNA and then delivering the coated particles through the skin into the underlying muscle with an air gun (called a *gene gun*). This will allow rapid delivery of a

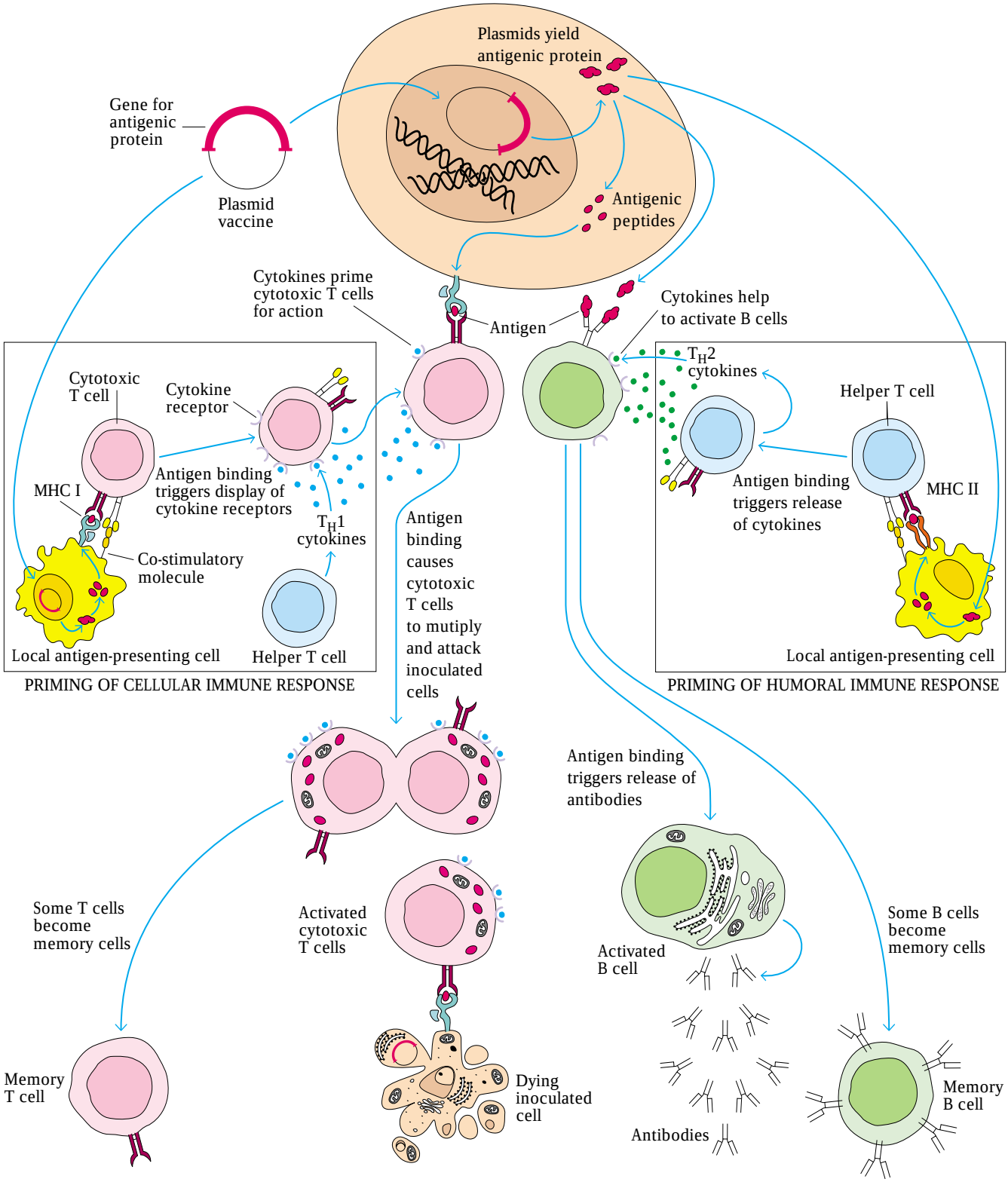


FIGURE 18-6 Use of DNA vaccines raises both humoral and cellular immunity. The injected gene is expressed in the injected muscle cell and in nearby APCs. The peptides from the protein encoded by the DNA are expressed on the surface of both cell types after processing as an endogenous antigen by the MHC class I pathway. Cells that present the antigen in the context of class I MHC molecules stimulate development of cytotoxic T cells. The protein encoded by the injected DNA is also expressed as a soluble, secreted protein, which is taken up, processed, and presented in the context of class II MHC molecules. This pathway stimulates B-cell immunity and generates antibodies and B-cell memory against the protein. *[Adapted from D. B. Weiner and R. C. Kennedy, 1999,* Sci. Am. ***281:****50.]*

vaccine to large populations without the requirement for huge supplies of needles and syringes.

Tests of DNA vaccines in animal models have shown that these vaccines are able to induce protective immunity against a number of pathogens, including the influenza virus. It has been further shown that the inclusion of certain DNA sequences in the vector leads to enhanced immune response. At present, there are human trials underway with several different DNA vaccines, including those for malaria, AIDS, influenza, and herpesvirus. Future experimental trials of DNA vaccines will mix genes for antigenic proteins with those for cytokines or chemokines that direct the immune response to the optimum pathway. For example, the IL-12 gene may be included in a DNA vaccine; expression of IL-12 at the site of immunization will stimulate TH_1-type immunity induced by the vaccine.

DNA vaccines will likely be used for human immunization within the next few years. However, they are not a universal solution to the problems of vaccination; for example, only protein antigens can be encoded—certain vaccines, such as those for pneumococcal and meningococcal infections, use protective polysaccharide antigens.

Multivalent Subunit Vaccines

One of the limitations of synthetic peptide vaccines and recombinant protein vaccines is that they tend to be poorly immunogenic; in addition, they tend to induce a humoral antibody response but are less likely to induce a cell-mediated response. What is needed is a method for constructing synthetic peptide vaccines that contain both immunodominant B-cell and T-cell epitopes. Furthermore, if a CTL response is desired, the vaccine must be delivered intra-cellularly so that the peptides can be processed and presented together with class I MHC molecules. A number of innovative techniques are being applied to develop multivalent vaccines that can present multiple copies of a given peptide or a mixture of peptides to the immune system (Figure 18-7).

VISUALIZING CONCEPTS

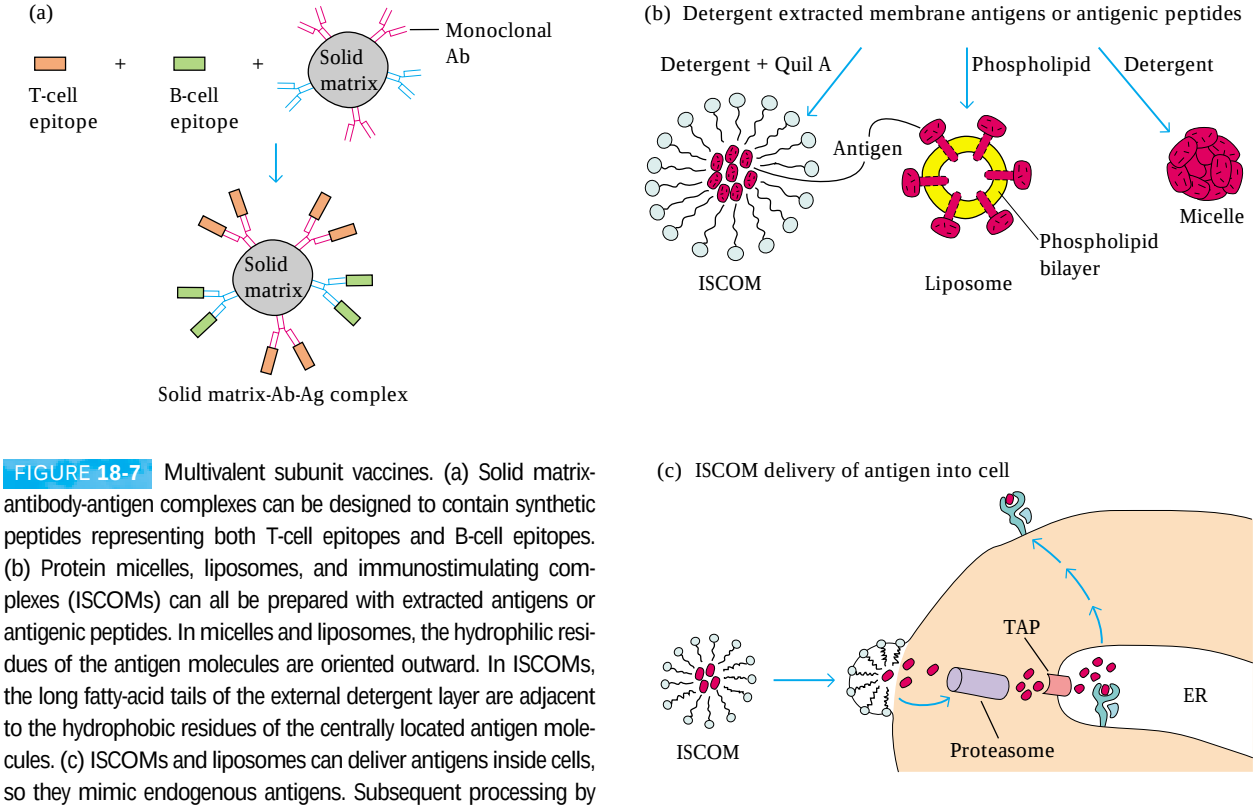


FIGURE 18-7 Multivalent subunit vaccines. (a) Solid matrix-antibody-antigen complexes can be designed to contain synthetic peptides representing both T-cell epitopes and B-cell epitopes. (b) Protein micelles, liposomes, and immunostimulating complexes (ISCOMs) can all be prepared with extracted antigens or antigenic peptides. In micelles and liposomes, the hydrophilic residues of the antigen molecules are oriented outward. In ISCOMs, the long fatty-acid tails of the external detergent layer are adjacent to the hydrophobic residues of the centrally located antigen molecules. (c) ISCOMs and liposomes can deliver antigens inside cells, so they mimic endogenous antigens. Subsequent processing by the cytosolic pathway and presentation with class I MHC molecules induces a cell-mediated response.

One approach is to prepare solid matrix–antibody-antigen (SMAA) complexes by attaching monoclonal antibodies to particulate solid matrices and then saturating the antibody with the desired antigen. The resulting complexes are then used as vaccines. By attaching different monoclonal antibodies to the solid matrix, it is possible to bind a mixture of peptides or proteins, composing immunodominant epitopes for both T cells and B cells, to the solid matrix (see Figure 18-7a). These multivalent complexes have been shown to induce vigorous humoral and cell-mediated responses. Their particulate nature contributes to their increased immunogenicity by facilitating phagocytosis by phagocytic cells.

Another means of producing a multivalent vaccine is to use detergent to incorporate protein antigens into protein micelles, lipid vesicles (called liposomes), or immunostimulating complexes (see Figure 18-7b). Mixing proteins in detergent and then removing the detergent forms micelles. The individual proteins orient themselves with their hydrophilic residues toward the aqueous environment and the hydrophobic residues at the center so as to exclude their interaction with the aqueous environment. Liposomes containing protein antigens are prepared by mixing the proteins with a suspension of phospholipids under conditions that form vesicles bounded by a bilayer. The proteins are incorporated into the bilayer with the hydrophilic residues exposed. Immunostimulating complexes (ISCOMs) are lipid carriers prepared by mixing protein with detergent and a glycoside called Quil A.

Membrane proteins from various pathogens, including influenza virus, measles virus, hepatitis B virus, and HIV have been incorporated into micelles, liposomes, and ISCOMs and are currently being assessed as potential vaccines. In addition to their increased immunogenicity, liposomes and ISCOMs appear to fuse with the plasma membrane to deliver the antigen intracellularly, where it can be processed by the cytosolic pathway and thus induce a cell-mediated response (see Figure 18-7c).

SUMMARY

- A state of immunity can be induced by passive or active immunization
  a) Short-term passive immunization is induced by transfer of preformed antibodies.
  b) Infection or inoculation achieves long-term active immunization.
- Three types of vaccines are currently used in humans: attenuated (avirulent) microorganisms, inactivated (killed) microorganisms, or purified macromolecules.
- Protein components of pathogens expressed in cell culture may be effective vaccines.
- Recombinant vectors, including viruses or bacteria, engineered to carry genes from infectious microorganisms, maximize cell-mediated immunity to the encoded antigens.
- Plasmid DNA encoding a protein antigen from a pathogen can serve as an effective vaccine inducing both humoral and cell-mediated immunity.
- Realizing the optimum benefit of vaccines will require cheaper manufacture and improved delivery methods for existing vaccines.

References

Afzal, M. F., et al. 2000. Clinical safety issues of measles, mumps, and rubella vaccines. *WHO Bull.* **78:**199.

Bloom, B. R. 1998. The highest attainable standard: ethical issues in AIDS vaccines. *Science* **279:**186.

DiTommaso, A., et al. 1997. Acellular pertussis vaccines containing genetically detoxified pertussis toxin induce long-lasting humoral and cellular responses in adults. *Vaccine* **15:**1218.

Dittmann, S., 2000. Sucessful control of epidemic diphtheria in the states of the former Union of Soviet Socialist Republics: lessons learned. *J. Inf. Dis.* **181** (Suppl. 1):S10.

Grandi, G. 2001. Antibacterial design using genomics and proteomics. *Trends in Biotech.* **19:**181.

Gurunathan, S., et al. 2000. DNA vaccines: immunology, application and optimization. *Ann. Rev. Immunol.* **18:**927.

Henderson, D. A. 1976. The eradication of smallpox. *Sci. Am.* **235:**25.

Shann, F., and M. C. Steinhoff. 1999. Vaccines for children in rich and poor countries. *Lancet.* **354**(Suppl. II):7.

Sutter, R. W., et al. 2000. Poliovirus vaccines: progress toward global poliomyelitis eradication and changing routine immunization recommendations in the United States. *Ped. Clinics of North America* **47:**287.

Takahashi, H., et al. 1990. Induction of $CD8^+$ cytotoxic T cells by immunization with purified HIV-1 envelope protein in ISCOMS. *Nature* **344:**873.

Weiner, D. B., and R. C. Kennedy. 1999. Genetic vaccines. *Sci. Am.* **281:**50.

USEFUL WEB SITES

http://www.VaccineAlliance.org/

Homepage of global alliance for vaccines and immunization (GAVI), a source of information about vaccines in developing countries and worldwide efforts at disease eradication.

http://www.ecbt.org/

Every Child by Two offers information on childhood vaccination.

Study Questions

CLINICAL FOCUS QUESTION A connection between the new pneumococcus vaccine and a relatively rare form of arthritis has been reported. What data would you need to validate this report? How would you proceed to evaluate this possible connection?

1. Indicate whether each of the following statements is true or false. If you think a statement is false, explain why.
   a. Transplacental transfer of maternal IgG antibodies against measles confers short-term immunity on the fetus.
   b. Attenuated vaccines are more likely to induce cell-mediated immunity than killed vaccines are.
   c. Multivalent subunit vaccines generally induce a greater response than synthetic peptide vaccines.
   d. One disadvantage of DNA vaccines is that they don't generate significant immunologic memory.
   e. Macromolecules generally contain a large number of potential epitopes.
   f. A DNA vaccine only induces a response to a single epitope.
2. What are the advantages and disadvantages of using attenuated organisms as vaccines?
3. A young girl who had never been immunized to tetanus stepped on a rusty nail and got a deep puncture wound. The doctor cleaned out the wound and gave the child an injection of tetanus antitoxin.
   a. Why was antitoxin given instead of a booster shot of tetanus toxoid?
   b. If the girl receives no further treatment and steps on a rusty nail again 3 years later, will she be immune to tetanus?
4. What are the advantages of the Sabin polio vaccine compared with the Salk vaccine? Why is the Sabin vaccine no longer recommended for use in the United States?
5. In an attempt to develop a synthetic peptide vaccine, you have analyzed the amino acid sequence of a protein antigen for (a) hydrophobic peptides and (b) strongly hydrophilic peptides. How might peptides of each type be used as a vaccine to induce different immune responses?
6. Explain the phenomenon of herd immunity. How does this phenomenon relate to the appearance of certain epidemics?
7. You have identified a bacterial protein antigen that confers protective immunity to a pathogenic bacterium and have cloned the gene that encodes it. The choices are either to express the protein in yeast and use this recombinant protein as a vaccine, or to use the gene for the protein to prepare a DNA vaccine. Which approach would you take and why?
8. Explain the relationship between the incubation period of a pathogen and the approach needed to achieve effective active immunization.
9. List the three types of purified macromolecules that are currently used as vaccines.