VACCINE TECHNOLOGY

A vaccine is a preparation used to prevent a specific infectious disease by inducing immunity in the host against a specific pathogenic microorganism. The practice of administering a vaccine is called immunization. The first human immunization was performed in 1796 by Edward Jenner in England, which led to the discovery of the smallpox vaccine. However, classic vaccinology developed 100 years later, after the work by Louis Pasteur demonstrated that microorganisms are causes of diseases.

During the early 1900s, vaccines against major human epidemic diseases such as pertussis, diphtheria, tetanus, and tuberculosis were developed. Vaccines for many animal diseases were also available. In the early 1950s, the development of cell culture techniques by J. E. Enders at Harvard was followed by another series of major advances in vaccine development. Vaccines against polio, mumps, measles, and rubella were licensed during the 1960s.

However, with the discovery and widespread use of antibiotics, beginning in the 1950s, the interest in vaccine research diminished. It was anticipated that infectious diseases would no longer be a threat to human health. In fact, since the licensure of polio in 1963, no other vaccines were licensed for infant primary series immunization until the introduction of *Hemophilus influenzae* type b conjugate vaccine in 1990. The development of biotechnology and modern immunology created new opportunities for producing new antigens, and vaccine research has become a primary focus in recent years. As a result, several vaccines have been approved, such as Hepatitis B, Hepatitis A, Acellular pertussis, Pneumococcal conjugate, Meningococcal conjugate, Rotavirus, Varicella, Measles-Mumps-Rubella with Varicella, Herpes Zoster, and Human Papilloma virus. This review focuses mainly on the U.S. market.

Preventive medicine through vaccination continues to be the most cost-effective public health practice (1) even with the drastic advance in modern medicine. Mass vaccination programs have eradicated smallpox from the Earth. The World Health Organization (WHO) has a major campaign underway to eradicate polio. The development of vaccines has saved millions of lives and prevented many more from suffering. However, there are still many diseases, such as malaria, without effective vaccines. With the recent emergence of antibiotic-resistance strains and exotic viruses, an effective vaccine development program becomes a top priority of public health policy.

1. Commercial Vaccines

Vaccines can be roughly categorized into killed vaccines and live vaccines. A killed vaccine can be (*1*) an inactivated, whole microorganism such as inactivated polio virus; (*2*) an inactivated toxin, called a toxoid, such as a diphtheria toxoid; or (*3*) one or more components of the microorganism commonly referred to as subunit vaccines. The examples are capsular polysaccharide of *Streptococcus pneumoniae* and the surface antigen protein for Hepatitis B virus vaccine.

Live vaccines are normally weakened strains that do not cause diseases in the host but can still stimulate the immune response. A typical example is the measles–mumps–rubella vaccine. The weakening of microorganisms or attenuation of the virus or bacteria can be accomplished by passage through different substrates and/or at different temperatures or by chemical mutagenesis. Modern genetic engineering techniques can also be used to attenuate a virus or bacterium.

Vaccines for human use are regulated by the Food and Drug Administration in the United States and Boards of Health in other countries. The manufacturing of vaccines requires adherence to strict current good manufacturing practices (cGMPs), and in the United States, both the process and the facility where the vaccine is produced must be licensed. The Center for Biologics Evaluation and Research is the branch of the FDA that regulates vaccines. Basic requirements are described in the *Code of Federal Regulations* (CFR) (2).

Vaccines are used in either the general population of children or adults or for special groups. Recommendations for vaccine usage are made by the Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control. The Committee on Infectious Diseases of the American Academy of Pediatrics (Redbook Committee) also makes recommendations for infants through adolescents, and the American Academy of Family Physicians makes recommendations for adults. An excellent review of vaccine history, development, usage, and related regulatory issues is available (3).

2. Vaccines for the General Population

Vaccines in this category protect children and adults from polio, diphtheria, tetanus, pertussis (whooping cough), measles (rubeola), mumps, rubella (German

measles), hepatitis B, hepatitis A, hemophilus disease (meningitis, epiglotitis), pneumococcal and menigococcal diseases, varicella (chicken pox), and influenza. The basic schedule for children and adolescents is given in Table 1 (4).

2.1. Poliomyelitis. Both live (attenuated) and killed vaccines are used in prevention of polio in children. In the United States, the killed or inactivated vaccine is recommended for immunization of children at 2, 4, 6–18 months and the fourth dose at 4–6 years.

Composition and Methods of Manufacture. This vaccine contains three types of polio virus. It is a highly purified, inactivated vaccine produced by microcarrier culture. This culture technique and improvements in purification, concentration, and standardization of poliovirus antigen produce a more potent and consistent immunogenic vaccine than prevous vaccine preparations (prior to 1998). The viruses are grown in cultures of Vero cells, a continuous line of monkey kidney cells, by microcarrier technique (5,6). The cells are grown in Eagle minimum essential medium modified medium, supplemented with newborn calf serum tested for adventitious agents prior to use. After clarification and filtration, viral suspensions are concentrated by ultrafiltration and purified by three liquid chromatography steps: one column of anion exchanger, one column of gel filtration, and again one column of anion exchanger. After re-equilibration of the purified viral suspension with Medium M-199 and adjustment of the antigen titer, the monovalent viral suspension is inactivated at 37°C for at least 12 days with 1:4000 formalin. Each dose of trivalent vaccine is formulated to contain 40-D antigen units of type 1, 8-D antigen units of type 2, and 32-D antigen units of type 3 poliovirus (7).

Standardization and Testing. For each lot of vaccine, D-antigen content is determined *in vitro* using the D-antigen enzyme-linked immunosorbent assay (ELISA) assay, and immunogenicity is determined by *in vivo* testing in animals. Passage levels of the attenuated vaccine strains are strictly controlled to ensure that the vaccine maintains its attenuated characteristics. The poliovirus genome has been cloned and sequenced, such that it is possible to maintain a cDNA repository and develop a better understanding of the biology of the vaccine viruses (8,9).

2.2. Diphtheria, Tetanus, and Pertussis. These vaccines, in combination, have been routinely used for active immunization of infants and young children since the 1940s. The development of acellular pertussis in the early 1990s has replaced the whole-cell pertussis vaccine. The recommended schedule in the United States calls for immunizations at 2, 4, and 6 months of age with boosters at 12–18 months and 4–6 years of age with DTaP vaccine. Note that the immumization schedules differ in other parts of the world. Since 1993, these vaccines have been available in combination with a vaccine that protects against *Hemophilus* disease, thus providing protection against four bacterial diseases in one preparation. A booster immunization with diphtheria and tetanus only is recommended once every 10 years after the fifth dose.

Composition and Methods of Manufacture. The diseases of diphtheria and tetanus are caused by toxins synthesized by the organisms *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively. Diphtheria and tetanus vaccines contain purified toxins that have been inactivated by formaldehyde to form toxoids.

Table 1. **Recommended Childhood and Adolescent Immunization Schedule—United States, 2006**[a]

Age► / Vaccine▼	Birth	1 mo	2 mo	4 mo	6 mo	12 mo	15 mo	18 mo	24 mo	4–6 yrs	11–12 yrs	13–14 yrs	15 yrs	16–18 yrs
Hepatitis B	HepB	HepB		HepB	HepB				HepB Series					
Diphtheria, Tetanus, Pertussis			DTaP	DTaP	DTaP		DTaP			DTaP	Tdap	Tdap		
Hemophilus influenzae type b			Hib	Hib	Hib	Hib								
Inactivated Poliovirus			IPV	IPV	IPV					IPV				
Measles, Mumps, Rubella						MMR				MMR	MMR			
Varicella						Varicella			Varicella					
Meningococcal							Vaccines within broken line are for selected population		MPSV4		MCV4	MCV4	MCV4	
Pneumococcal			PCV	PCV	PCV	PCV			PCV	PPV				
Influenza					Influenza (yearly)				Influenza (yearly)					
HepatitisA						HepA Series			HepA Series					

Range of recommended ages.

Catch-up immunization.

Abbreviations: MCV4 = meningococcal conjugate vaccine; MPSV4 = meningococcal polysaccharide vaccine; PCV = pneumococcal conjugate vaccine; PPV = pneumococcal polysaccharide vaccine; Tdap = tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis.

[a]Ref. 4.

Tetanus toxin can be obtained by growing *Clostridium tetani* in a complex medium especially formulated for production of high yields of toxin. The medium contains complex nutrients such as an enzymatic digest of casein, dextrose, sodium chloride, and other essential nutrients (10,11). The medium for growth of the diphtheria organism is also a complex liquid (12). The toxins of these bacteria are liberated into the growth medium and harvested away from intact cells and debris by filtration. The toxins may then be further purified, toxoided (or toxoided first), and followed by purification. Toxoiding involves incubation with formaldehyde at specified conditions of time, temperature, and pH. Purification can be accomplished by alcohol (methanol, acid pH) fractionation (13) or separation by serial ammonium sulfate fractionation from the culture fluid, or by column chromatography (13,14).

The acellular pertussis vaccines are prepared by a variety of methods that begin with growth of the bacteria in a complex medium, followed by chemical extraction and purification of the desired components away from the medium and cell debris. Formaldehyde treatment is used to inactivate any remaining toxic activity. Acellular pertussis vaccine contains a combination of the following proteins: filamentous hemagglutinin, pertussis toxoid, pertactin, and fimbriae types 2 and 3 (15–18). Not all components are present in several vaccines in use.

The final vaccine contains the two toxoids, as well as acellular pertussis antigens, a buffer, and an adjuvant, ie, a substance that increases the response to an antigen when combined with the antigen, eg, aluminum. As noted, the final vaccine can also contain a component that protects against *Hemophilus influenzae* b disease (19).

Standardization and Testing. Both dipththeria and tetanus toxoids induce at least two units of antitoxin per milliliter in the guinea pig potency test. The potency of the acellular pertussis vaccine components is evaluated by antibody response of immunized mice to pertussis toxin, filamentous hemagglutinin, pertactin, and fimbriae types 2 and 3 measured by ELISA (17).

2.3. Measles, Mumps, Rubella. Live, attenuated vaccines are used for simultaneous or separate immunization against measles, mumps, and rubella in children from 12 to15 months of age to puberty. Two doses, one at 12–15 months of age and the second at 4–6 or 11–18 years are recommended in the United States.

Composition and Methods of Manufacture. The combined vaccine for simultaneous immunization in the United States is a mixture of the three live, attenuated viruses: measles (Enders' attenuated Edmonston strain), mumps (Jeryl Lynn strain), and rubella (RA27/3 strain). Other strains of each of these viruses are used throughout the world. The measles and mumps viruses are propagated in cultures of primary chick embryo cells, whereas rubella is propagated in the WI-38 strain or the MRC-5 strain of human diploid cells. In either case, the cells are propagated and the viruses are grown in a tissue culture medium. After sufficient viral replication, the fluids are collected, clarified, and mixed together in the proper proportions along with stabilizers such as gelatin, sorbitol, and amino acids. The vaccine is presented in freeze-dried vials and must be reconstituted with sterile distilled water before injection (20,21).

Standardization and Testing. Potency is determined by titration of the amount of live virus in susceptible tissue culture and is run in parallel with a U.S. standard. Both *in vivo* and *in vitro* tests are used to assess safety (21).

2.4. *Hemophilus influenzae* Serotype b. Three vaccines are available for immunizing infants. Two of these vaccines are administered at 2, 4, and 6 months of age with a booster given at 12–15 months of age, and the third vaccine is administered at 2 and 4 months of age with a booster at 12–15 months of age.

Composition and Methods of Manufacture. The vaccines suitable for immunization of infants are all forms of the capsular polysaccharide of the *H. influenzae* b strain conjugated to a carrier protein. The antibodies generated to the capsule are protective. The carrier proteins are either CRM_{197} (a naturally nontoxic variant of diphtheria toxin), tetanus toxoid, or an outer membrane protein from the *Neisseria meningitidis* bacterium. Manufacturing is accomplished by separate process streams for fermenting and purifying the carrier protein and the capsular polysaccharide. The polysaccharide may be cleaved into smaller units before conjugation to the carrier protein. Conjugation is accomplished by activating either the protein or the saccharide and joining with or without a linking agent (22–25). This vaccine can be combined with DTaP as a combination vaccine.

Standardization and Testing. Requirements are generally specified within licenses in the United States and include a variety of in-process tests to assess purity, safety, and potency of the individual components and potency and safety of the final product. Potency is standardized by determining the size of the conjugate and the quantitative amount of saccharide that is bound to the carrier protein. General safety and immunogenicity are assessed in animals.

2.5. Hepatitis B. Routine vaccination of all children and adolescents is recommended because a major part of the disease burden of Hepatitis B (Hep B) is due to the large number of Hep B infections that occur in children. Infants receive immunizations at birth, 1–2 months, and a third dose at 6 to 18 months. Other schedules are available for immunization of adolescents and adults who have not received the vaccine.

Composition and Methods of Manufacture. Hepatitis B vaccines consist predominantly of 22-nm particles of the S antigen of the Hepatitis B. Some vaccines also include varying amounts of pre-S1 and/or pre-S2 antigens that are precursors to the fully matured surface antigen of this virus. The antigen can be derived from the plasma of chronic carriers using plasma fractionation techniques that ensure purity and inactivation of any unwanted live agents or, more commonly, from recombinant organisms. There are systems using recombinants of yeasts or Chinese hamster ovary (CHO) cells that can be used to produce the surface antigen on a large scale (26–28).

Standardization and Testing. Potency is determined by quantitating the Hepatitis B surface antigen by an antibody-binding assay combined with a determination of the amount of protein. Safety testing typical for cell culture-derived products is also performed and includes assuring the absence of live virus.

2.6. Varicella. The varicella (chicken pox) vaccine was approved for immunization of children. A single dose at one year of age is recommended. The vaccine is also available in combination with measles, mumps, and rubella (29).

Composition and Methods of Manufacture. Vaccine is produced from the Oka attenuated strain. Vaccine is produced in human diploid cells such as MRC-5. After growth in the cell substrate, the cells are harvested into the growth medium and sonicated to release the cell-associated virus. Sucrose and buffering salts are generally in the medium to help stabilize the virus. The vaccine is presented in a freeze-dried vial to be reconstituted with sterile distilled water before injection (30).

Standardization and Testing. Potency is determined by titrating the amount of live virus using a suitable cell substrate. Safety testing is also performed on seed lots to assure proper attenuation and on vaccine to assure absence of unwanted contaminants.

2.7. Pneumococcus. Two vaccines are licensed to prevent pneumococcal infections. The unconjugated, 23-valent pneumococcal polysaccharide vaccine that is recommended for the adult population is discussed later in this review. The other is a conjugate vaccine that is recommended for vaccination of infants and children. The pneumococccal conjugate vaccine was licensed for prevention of invasive disease in infants and children caused by *Streptococcus pneumoniae*. The vaccine also indicated for active immunization of infants and toddlers against otitis media caused by serotypes included in the vaccine. The recommended schedule calls for routine immunization at 2, 4, 6, and 12–15 months of age.

Composition and Methods of Manufacture. The vaccine is a sterile solution of saccharides of the capsular antigens of *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F individually conjugated to diphtheria CRM_{197} protein. Each serotype is grown in soy peptone broth. The individual polysaccharides are purified through centrifugation, precipitation, ultrafiltration, and column chromatography. The polysaccharides are chemically activated prior to conjugation to the carrier protein CRM_{197} to form the glycoconjugate. This is effected by reductive amination. CRM_{197} is a nontoxic variant of dipththeria toxin isolated from cultures of *Corynebacterium diphtheriae* strain C7 [197] grown in a casamino acids and yeast extract-based medium. CRM_{197} is purified through ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography. The individual glycoconjugates are purified by ultrafiltration and column chromatography, and they are analyzed for saccharide-to-protein ratios, molecular size, free saccharide, and free protein. The vaccine is manufactured as a liquid preparation. Each 0.5-mL dose contains 2 mcg of each saccharide for serotypes 4, 9V, 14, 18C, 19F, and 23F and 4 mcg of serotype 6B per dose; and approximately 20 mcg of CRM_{197} carrier protein; and 0.125 mg of aluminum per 0.5-mL dose as aluminum phosphate adjuvant (31).

Standardization and Testing. The individual glycoconjugates are compounded to formulate the vaccine. The potency of the formulated vaccine is determined by quantitation of each of the saccharide antigens and by the saccharide-to-protein ratios in the individual glycoconjugates.

2.8. Hepatitis A. Hepatitis A virus is one of the several hepatitis viruses that cause a systemic infection with pathology in the liver. The incubation period ranges from approximately 20 to 50 days. This vaccine is available for both pediatric and adult vaccinations and is indicated for active immunization of persons $\geq$12 months of age.

Composition and Methods of Manufacture. The inactivated whole virus vaccine is derived from the hepatitis A virus grown in cell culture in MRC-5 human diploid cells. After removal of the cell culture medium, the virus suspension is purified through a combination of physical and high-performance liquid chromatography or ultrafiltration and gel permeation procedures. The virus is inactivated with formalin and adsorbed onto aluminum adjuvant. One milliliter of the vaccine contains approximately 50 units of hepatitis A virus antigen (33). The potency of vaccine is also expressed in terms of ELISA Units (EL. U), and each milliliter consists of 1440 EL. U. (32). The pediatric dose is 0.5 mL, whereas the adult dose is 1 mL. The 50U dose vaccine contains less than 0.1 mcg of nonviral protein, less than 4×10^{-6} mcg of DNA, less than 10^{-4} mcg of bovine alubumin, and less than 0.8 mcg of formaldehyde (33).

Standardization and Testing. The potency of the vaccine is determined by protein assay for hepatitis A virus antigen (50U per mL) or by ELISA assay (1440 EL.U. per mL).

3. Vaccines for Special Populations and Recently Approved Vaccines

Vaccines for special populations are listed in Table 2.

Two vaccines that are in fairly widespread use in the adult population are vaccines that prevent viral influenza and pneumococcal pneumonia. Meningococcal conjugate vaccine, Rotavaccine, herpes zoster vaccine, and human papilloma vaccine have recently been approved.

3.1. Influenza (Inactivated). Influenza is a significant cause of death and, along with pneumonia, is the seventh leading cause of death across generations (44). This understates the actual impact of influenza as the complications associated with influenza infection are also categorized as heart disease,

Table 2. **Selectively Used Vaccines**

Type	Composition	Use	Reference
	Viral		
Rabies	Inactivated rabies grown in culture of human diploid cells	Post-exposure for treatment of animal bites, pre-exposure for those at high-risk	34–36
Yellow fever	Live, attenuated virus grown in embryonated chicken eggs	International travel to high risk-areas	37
	Bacterial		
Meningitis	Purified capsular polysaccharides of *Neisseria meningitidis* serogroups A, C, Y, W 135	Control outbreaks and for military recruits	38–40
Typhoid	Inactivated whole cells, capsular polysaccharide, or live attenuated bacteria	International travel to high-risk areas	41
Plague	Inactivated *Yersinia pestis*	High risk of exposure	42
Tuberculosis	Bacille Calmette Guerin	High-risk groups	43

chronic lower respiratory disease, or diabetes. As a result, influenza each year conservatively contributes, in the United States, to over 36,000 deaths, many of which could be prevented through vaccination. Influenza viruses can also cause pandemics during which rates of illness and death from influenza-related complications can increase dramatically. Influenza viruses cause disease among all age groups. Rates of infection are highest among children, but rates of serious illness and death are highest among persons of $\geq$65 years and persons of any age who have medical conditions that place them at increased risk for complications from influenza (45). The ACIP recommends annual influenza vaccination for all persons who are at risk from infections of the lower respiratory tract and for all older persons. Total of three influenza viruses (two of type A and one of type B) are responsible for periodic outbreaks of febrile respiratory disease. Influenza A viruses are further categorized into subtypes based on two surgace antigens: hemagglutinin and neuraminidase. Influenza B viruses are not categorized into subtypes. New influenza virus variants result from frequent anatigenic change (ie, antigenic drift), resulting from point mutations that occur during viral replication. Influenza B viruses undergo antigenic drift less rapidly than influenza A viruses.

Composition and Methods of Manufacture. The manufacture of an effective vaccine is complicated by antigenic variation or drift, which can occur from year to year within the virus types, making the previous year's vaccine less effective. Each year, antigenic characterization is important for selecting the virus strains to be included in the vaccine.

Vaccines are prepared by growing high-yielding strains of influenza viruses in embryonated chicken eggs. The viruses are harvested and inactivated with formaldehyde. Influenza virus is concentrated and purified in a linear density gradient solution using a continuous flow centrifuge. The virus is then chemically disrupted usng a nonionic surfactant, Triton X-100, producing a "split virus." The split virus is further purified by chemical means and suspended in sodium phosphate-buffered isotonic sodium chloride solution (46).

Standardization and Testing. The final vaccine is tested for safety, potency, and residual chemicals. Safety includes testing for endotoxin and sterility. Potency is evaluated by quantitative determination of the amount of hemagglutinin in the vaccine. An antibody to this glycoprotein is associated with protection. The single radial immunodiffusion (SRID) technique is used to standardize the mass of this protein in comparison with a reference preparation.

3.2. Influenza (Live, Intranasal). The vaccine is indicated for the prevention of disease caused by influenza A and B viruses in healthy children and adolescents, 5–17 years of age, and healthy adults, 18–49 years of age.

Composition and Methods of Manufacture. Each 0.5-mL dose is formulated to contain 10 $TCID_{50}$ of live attenuated influenza virus assortants of the strains recommended by the U.S. Public Health Service. These strains are (*1*) antigenically representative of influenza viruses that may circulate in humans; (*2*) cold-adapted, ie, they replicate efficiently at 25°C, a temperature that is restrictive for replication of many wild-type viruses; (*3*) temperature-sensitive, ie, they are restricted in replication at 37°C or 39°C, temperatures at which many wild-type influenza viruses grow efficiently; and (*4*) attenuated, so as not to produce classic influenza-like illness in the ferret model of human

influenza infection. The cumulative effect of these changes is that the attenuated vaccine viruses replicate in the nasopharynx to induce protective immunity. Each of the three influenza virus strains contained in the vaccine is a genetic reassortant of a Master Donor Virus and a wild-type influenza virus. Viral harvests used in the production of the vaccine are produced by inoculating each of the three reassortant viruses into specific pathogen-free (SPF) eggs that are incubated to allow for virus replication. The allantoic fluid of these eggs is harvested, clarified by centrifugation, and stabilized with buffer containing sucrose, potassium phosphate, and monosodium glutamate. Viral harvests from the strains are subsequently blended and diluted as required to desired potency with allantoic fluid derived from uninfected SPF eggs and/or diluted stabilizing buffer to produce trivalent bulk (47).

Standardization and Testing. Each lot of viral harvest is tested for (*1*) cold-adaptation, (*2*) temperature-sensitivity, and (*3*) attenuation, and tested extensively by *in vitro* and *in vivo* methods to detect adventitious agents.

3.3. Pneumococcal Polysaccharide. The pneumococcal polysaccharide vaccine may be used for immunization of persons two years of age or older who are at increased risk of pneumococcal disease. However, the vaccine is predominatly used in elderly populations. Note that this vaccine is an unconjugated, polysaccharide vaccine. The conjugated pneumococcal polysaccharide vaccine recommended for infants and children was discussed earlier in this review.

Composition and Methods of Manufacture. The vaccine consists of a mixture of purified capsular polysaccharides from 23 pneumococcal types that are responsible for over 90% of the serious pneumococcal disease in the world (48–50). Each polysaccharide type is produced separately and treated to remove impurities. The latter is commonly achieved by alcohol fractionation, centrifugation, treatment with cationic detergents, proteolytic enzymes, nucleases, diafiltration, and lyophilization (51). The vaccine contains 25 mcg of each pneumococcal polysaccharide and 0.25% phenol as a preservative (50).

Standardization and Testing. The Center for Biologics Evaluation and Research has set guidelines for the vaccine that include standards for size of the individual polysaccharides and specifications for both purity (absence of protein and nucleic acid) and chemical and immunological identity.

3.4. Meningococcus. The meningococcal conjugate vaccine is indicated for active immunization of adolescents and adults 11–55 years of age for the prevention of invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, Y, and W-135. Note that conjugate vaccines against *Neisseria meningitidis* serogroup C alone are licensed outside of the United States.

Composition and Methods of Manufacture. Each 0.5-mL dose of vaccine is formulated in sodium phosphate-buffered isotonic sodium chloride solution to contain 4 mcg each of meningococcal A, C, Y, and W-135 polysaccharides conjugated to approximately 48 mcg of diphtheria toxoid protein carrier. *N. meningitidis* A, C, Y, and W-135 stains are cultured on Mueller Hinton agar and grown in Watson Scherp media. The polysaccharides are extracted from *N. meningitidis* cells and purified by centrifugation, detergent precipitation, alcohol precipitation, solvent extraction, and diafiltration. To prepare polysaccharides for conjugation, they are depolymerized, derivatized, and purified by diafiltration. *Corynebacterium diphtheriae* cultures are grown in a modified Mueller and Miller medium and

detoxified with formaldehyde. The diphtheria toxoid protein is purified by ammonium sulfate fractionation and diafiltration. The derivatized polysaccharides are covalently linked to diphtheria toxoid and purified by serial diafiltration. The four meningococcal components, present as individual serogroup-specific glycoconjugates, compose the final formulated vaccine (52).

Standardization and Testing. The potency of the vaccine is determined by quantifying the amount of each polysaccharide antigen that is conjugated to diphtheria toxoid protein and the amount of unconjugated polysaccharide present.

3.5. Rotavirus. This live, oral, pentavalent vaccine contains five live reassortant rotaviruses. The rotavirus parent strains of the reassortants were isolated from human and bovine hosts. The vaccination series consists of three ready-to-use liquid doses of vaccine administered orally starting at 6 to 12 months of age, with subsequent doses administered at 4- to 10-week intervals. The third dose should not be given after 32 weeks of age. The viral strains replicate in the small intestine and induce immmunity.

Composition and Methods of Manufacture. Four reassortant rotaviruses express one of the outer capsid proteins (G1, G2, G3, or G4) from the human rotavirus parent stain and the attachment protein (P7) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein, P1A (genotype P[8]), from the human rotavirus parent strain and the outer capsid protein G6 from the bovine rotavirus parent strain. The reassortants are propagated in Vero cells using standard cell culture techniques in the absence of antifungal agents. The reassortants are suspended in a buffer stabilizer solution. Each vaccine dose contains sucrose, sodium citrate, sodium phosphate, sodium hydroxide, polysorbate 80, cell culture media, and trace amounts of fetal bovine serum (53).

Standardization and Testing. The minimum dose levels (10^6 infectious units) range from 2.0 to 2.8, depending on the reassortant.

3.6. Herpes Zoster. Herpes Zoster, commonly known as shingles or zoster, is a manifestation of the reactivation of varicella-zoster virus (VZV), which as a primary infection, produces chickenpox (varicella). After initial infection, the virus remains latent in dorsal root or cranial sensory ganglia until it reactivates, producing zoster. Zoster is characterized by a unilateral, painful, vesicular cutaneous eruption with dermatomal distribution. The incidence and severity of herpes zoster and postherpetic neuralgia increase with age in association with a progressive decline in cell-mediated immunity to VZV. The vaccine is indicated for prevention of herpes zoster in individuals 60 years of age and older (54).

Composition and Methods of Manufacture. The vaccine is a lyophilized preparation of the Oka/Merck strain of live, attenuated varicella-zoster virus. The virus was initially obtianed from a child with naturally occurring varicella, then introduced into human embryonic lung cell cultures, adapted to and propagated in embryonic guinea pig cell cultures, and finally propageted in human diploid cell cultures. Further passage was performed in human diploid cell cultures. The cells, virus seeds, virus bulks, and bovine serum used in the manufacturing process are all tested to provide assurance that the final product is free of adentitious agents (54).

Standardization and Testing. Each dose (0.65-mL) contains a minimum of 19,400 plaque-forming units of Oka/Merck stain of VZV when reconstituted and stored at room temperature for up to 30 minutes. Each dose contains sucrose, hydrolyzed porcine gelatin, sodium chloride, monosodium L-glutamate, and other salts. Residual components of mamamlian cells include DNA and protein.

3.7. Human Papilloma Virus. Two vaccine candidates have been developed to reduce the acquisition and clinical disease by common human papilloma virus (HPV) types. One is currently licensed in the United States. HPV causes squamous cell cervical cancer and cervical adenocarcinoma. HPV also causes approximately 35–50% of vulvar and vaginal cancers. It also causes genital warts. HPV 6, 11, 16, and 18 are common HPV types. The vaccine is indicated in girls and women 9 to 26 years of age for the prevention of the diseases caused by HPV types 6, 11, 16, and 18. The vaccine is administered as three separate 0.5-mL doses acccording to the following schedule: at elected date, 2 months, and 6 months after the first dose (55).

Composition and Methods of Manufacture. The vaccine is a noninfectious recombinant, quadrivalent vaccine prepared from the highly purified virus-like particles (VLPs) of the major capsid (L1) protein of HPV Types 6, 11, 16, and 18. The L1 Proteins are produced by separate fermentations in recombinant *Saccharomyces cerevisiae* and self-assembled into VLPs. The fermentation process involves growth of *S. cerevisiae* on chemically defined fermentation media that include vitamins, amino acids, mineral salts, and carbohydrates. The VLPs are released from the yeast cells by cell disruption and purified by a series of chemical and physical methods. The purified VLPs are adsorbed on preformed amorphous aluminum hydroxyphosphate sulfate. The quadrivalent HPV VLP vaccine is a sterile liquid suspension that is prepared by combining the adsorbed VLPs of each HPV type (55).

Standardization and Testing. Each 0.5-mL dose of vaccine contains approximately 20 mcg of HPV 6 L1 protein, 40 mcg of HPV 11 L1 protein, 40 mcg of HPV 16 L1 protein and 20 mcg of HPV 18 L1 protein.

4. Vaccines Being Developed

Despite the tremendous advances since the 1960s in the biomedical fields, including the total eradication of smallpox and reduction of mortality resulting from various diseases, a large number of diseases remains that are endemic in many parts of the world. The developing countries bear the brunt of several of these, eg, malaria, trypanosomiasis, and schistosomiasis. In developed countries, diseases such as herpes and gonorrhea are becoming increasingly prevalent. Vaccines for many etiological agents that still cause disease have not been manufactured for several reasons. These reasons include a lack of understanding of how immunity can be artificially induced and an inability to grow sufficient quantities of these agents to produce vaccines. However, new vaccines have been developed and licensed, whereas good progress is advancing in other areas. In the meantime, emerging exotic viruses such as HIV and drug-resistant pathogens continue to appear. There is an urgent need to expand vaccine R&D to reduce the risk of disease in the future.

4.1. Group B Meningococcus. The capsular polysaccharide of group B meningococcus is not immunogenic in humans. Thus, a conjugate vaccine of the group B polysaccharide will not improve its efficacy, and this remains a major challenge in developing the vaccine against group B organisms. One approach to improve the immunogenicity is to modify the polysaccharide (56). Another approach is to use the outer membrane proteins of the bacteria. Because of the different serotypes, a multivalent vaccine will be needed, if outer membrane proteins are to be used as the vaccine (57). The outer membrane protein preparation is normally a crude complex mixture or vesicle, or it can be a purified subunit protein. Both approaches have been in clinical trials (58,59). However, it is not anticipated that this vaccine will be in general use in the near future. Another approach is to use the lipooligosaccharide (LOS) of the bacteria as the antigen, which is a component of the bacterial endotoxin and conserved in all serotypes. A conjugate vaccine based on the LOS is being developed (60).

4.2. Respiratory Syncytial Virus. Respiratory syncytial virus (RSV) causes severe lower respiratory tract disease in infants. It is the major cause of pediatric hospitalization in the United States (~90,000 events/year), and it has a high mortality rate in neonates and other high-risk populations, such as the geriatric population. Development of an RSV vaccine has always been a major priority; however, earlier attempts have mostly failed (56).

Subunit, attenuated, and DNA vaccine approaches are being developed for RSV. A candidate subunit vaccine based on the surface (F) protein is being tested. The vaccine is prepared by infecting an appropriate cell substrate, such as Vero cells, which then expresses the F-protein on its surface. After the cells lyse, the protein is purified by chromatographic procedures. Clinical trials for both infants and the elderly are in progress. Another approach to protect the infant is passive immunization, through immunization of pregnant women. This maternal immunization of pregnant women with the subunit vaccine candidate is also being proposed (61).

Live attenuated vaccines for RSV are also being developed. Most of these vaccine candidates are derived from cold adaptation, by passing the virus at progressively lower temperatures than human body temperatures. However, other means of mutagenesis have been used to generate vaccine candidates (62). Several clinical trials of these vaccines are also in progress (63,64).

4.3. Parainfluenza. Parainfluenza viruses (PIV) also causes viral pneumonia in infants. It is similar to RSV; therefore, similar approaches are being used for developing a vaccine. A live attenuated PIV-3 vaccine has been in clinical trial (64).

4.4. Herpes Simplex. There are two types of herpes simplex virus (HSV) that infect humans. Type 1 causes orofacial lesions, and 30% of the U.S. population suffers from recurrent episodes. Type 2 is responsible for genital disease, and anywhere from 3×10^4–3×10^7 cases per year (including recurrent infections) occur. The primary source of neonatal herpes infections, which are severe and often fatal, is the mother infected with type 2. In addition, there is evidence to suggest that cervical carcinoma may be associated with HSV-2 infection (65,66).

Vaccine development is hampered by the fact that recurrent disease is common. Thus, natural infection does not provide immunity, and the best method to

induce immunity artificially is not clear. The genome of these viruses can cause transformation of normal cells, thus conferring on them one of the properties attributed to cancerous cells. Vaccine made from herpes viruses must, therefore, be carefully purified and screened to eliminate the possibility of including any active genetic material.

Vaccine candidates are based on the two viral surface proteins: gD and gB (66). Recombinant methods are used to express the proteins, either in Chinese hamster ovary (CHO) cells or in baculovirus. The proteins are purified as subunits and formulated with different adjuvants. Clinical trials with these vaccine candidates have been performed, but the results to date have not been encouraging.

A much better understanding of the pathogenesis of the virus and virus–host interactions are required for the efficient development of the vaccine. Recently, DNA immunization is being proposed as a means to stimulate the appropriate Th 1 response that might provide long-term protection (67).

4.5. Malaria. Malaria infection occurs in over 30% of the world's population and almost exclusively in developing countries. Approximately 150×10^6 cases occur each year, with one million deaths occurring in African children (68). The majority of the disease in humans is caused by four different species of the malarial parasite. Vaccine development is problematic for several reasons. First, the parasites have a complex lifecycle. They are spread by insect vectors and go through different stages and forms (intracellular and extracellular; sexual and asexual) as they grow in the blood and tissues (primarily liver) of their human hosts. In addition, malaria is difficult to grow in large quantities outside the natural host (69). Despite these difficulties, vaccine development has been pursued for many years. An overview of the state-of-the-art is available (70).

One of the early vaccine candidates was directed against sporozoites, the form of the parasites that is first injected into the host by a mosquito. With recent development of recombinant techniques, several circumsporozoite proteins or its related peptides were proposed as the vaccine candidates. Clinical trials have been carried out. The vaccines were immunogenic, but they did not provide sufficient protective efficacy (71,72).

Interest in vaccine development has centered around the asexual erythrocytic stage of the lifecycle, especially the merozoite. Several proteins associated with these stages have been identified and produced by recombinant techniques (73,74). The most prominent is the MSA-1 protein of the merozoite. A clinical trial with this protein is being planned (74).

The most advanced of all malaria vaccine candidates is SPf66 (75,76). It is a synthetic polypeptide. The peptide represents several protective epitopes correlated to several proteins of the pre-erythrocytic and asexual blood stage of *Plasmodium falciparum*. Extensive clinical trials with this vaccine have been carried out in South America and Africa (76–78). The efficacy of the vaccine varied in different regions and was generally lower than expected in a developed country. Consequently, the general application of the vaccine still generates much debate (79). However, this vaccine represents a major advance in the development of a malaria vaccine.

4.6. Gonorrhea. Gonorrhea, caused by *Neisseria gonorrhoeae*, is the most commonly reported communicable disease in the United States. Approximately 10^6 cases were reported to the Center for Disease Control (CDC) in

1979, but actual cases could be two to three times higher (80,81). In addition, an increasing number of strains is becoming resistant to penicillin, the antibiotic that is usually used to treat this disease.

Development of a vaccine is problematic because natural infection does not necessarily provide immunity. Whether this results from a poor immunological response or due to strain differences is not certain. Studies are being carried out on various structural components of the gonococcal bacterium, including pili, outer membrane proteins, lipopolysaccharide, and the outer capsule, in an effort to develop a vaccine (81). One of the more promising approaches involves a vaccine made with pili. These structures are responsible for attachment of the gonococci to mucosal surfaces, the first step necessary for infection to occur. Antisera against pili may prevent disease by preventing this attachment. One method for obtaining pili involves growth of the gonococci in liquid culture followed by mechanical shearing of the pili from the surface of the bacterium (82). Pili are further purified by differential centrifugation and ammonium sulfate precipitations. This type of preparation was shown to yield a protein pili vaccine that is immunogenic in human volunteers (83). Additional human studies indicate that a pili vaccine stimulates mucosal antibody formation (84).

4.7. Human Immunodeficiency Virus. Human immunodeficiency virus (HIV) causes Acquired Immunodeficiency Syndrome (AIDS), which has no cure. HIV infects the cells of the human immune system, such as T-lymphocytes, monocytes, and macrophages. After a long period of latency and persistent infection, it results in the progressive decline of the immune system and leads to full-blown AIDS, resulting in death.

Since the discovery of HIV-1 as a causative agent for AIDS, the development of vaccine against HIV-1 has been a top priority of the national public health agencies and medical research institutes. After 25 years of extensive research, there is much better understanding of the physiology and pathogenesis of the virus and host–virus interactions and responses. However, the effort in developing a vaccine has not been as successful as expected. The main problem is the tremendous antigenic variability of the virus (85). An antigen derived from the cultured strain might not be the same as the clinical strain. Another problem is the fact that the virus infects the cells of the human immune system, making the design of the vaccine more complex. It will require certain combinations of immune responses to provide long-term protection or eliminate the virus from the host. So far, the proper immune mechanism for achieving this goal has not been identified, although it is generally agreed that a cell-mediated immune response (CMI) is essential.

Until the 1990s, most of the vaccine candidates were derived from the surface proteins of the virus. HIV-1 envelope glycoproteins gp120 and gp160 have been extensively studied. Peptides or polypeptides related to these proteins are also being studied. Although these candidates all show immunogenicity and are protective in animal models, clinical studies of these proteins have not been able to demonstrate protection against the disease (86–88). With the disappointing outcomes from several clinical trials, the National Institute of Allergy and Infectious Diseases (NIAID) has decided to stop planning for any further phase III trials of any candidate vaccines (89). However, efforts

in development of the vaccine are being continued in the public and private research institutes.

5. Other Vaccines

Many other diseases do not have effective vaccines. These diseases are mostly regional in nature, epidemic in the developing world. Vaccines against parasites are also becoming critical to public health. Vaccines are being developed for dengue, Equine encephalitis, Tick-borne encephalitis, cholera, shigella, schistosomiasis, and SARS.

6. Future Technology

Vaccines for many diseases are unavailable because of an inability to determine the appropriate method for vaccination or difficulty in obtaining large quantities of antigens. Advances in medical science and immunology have substantially improved the understanding of the design and delivery of antigens. Genetic engineering offers further advances in providing the techniques for construction and production of large quantities of antigens. Development of these fields has been responsible for the rapid advances of vaccinology. Development of new vaccines also requires different process technology for the production of antigens and preparation of delivery system for vaccines. Other challenges for widespread use of vaccines include the development of cold chain and stability of vaccines at room temperature as well as low-cost vaccines for developing countries.

7. Immunology

Immunology is the basis of vaccine technology. Only through the better understanding of the function of the human immune system can better antigens as vaccine candidates be designed. For example, the discovery of the functions of T- and B-lymphocytes led to the development of capsular saccharide–protein conjugate vaccines. Discovery of the different Th 1 and Th 2 immune responses also generated great interest in designing a vaccine that can stimulate a specific immune response, which may be critical for some viral vaccines. CMI has also been demonstrated to be critical for a successful vaccine. Several vaccine candidates, especially for viral vaccines, have been based on this approach. The mucosal and secretory immune system has also been studied extensively. This area has led to a better design of vaccines for oral delivery or intranasal delivery.

8. Genetic Engineering

Genetic engineering (recombinant DNA technology) has been reviewed (56,90–94). It involves preparation of DNA fragments (passengers) coding for the substance of interest, inserting the DNA fragments into vectors (cloning vehicles), and introducing the recombinant vectors into living host cells where the passenger

DNA fragments replicate and are expressed, ie, transcribed and translated, to yield the desired substance. The passenger DNA fragments can be obtained from natural DNA molecules by treatment with restriction endonucleases (enzymes that cut DNA at specific sites) or by mechanical shearing (95,96). They can also be synthesized either from messenger RNA (mRNA), through the actions of reverse transcriptase and DNA polymerase, or by pure chemical methods (97–103). The vectors are autonomously replicating DNA molecules (replicons), eg, plasmids, bacteriophages, and animal viruses. Small plasmids and bacteriophages are the most suitable vectors because their maintenance does not require integration into the host genome and their DNA can be isolated readily in an intact form (94). Many plasmid and bacteriophage vectors of improved qualities have been constructed by addition to and deletion of some of their genetic elements. Insertion of passenger DNA fragments into cloning vehicles can be carried out by one of three methods: ligation of cohesive ends produced by restriction endonuclease, homopolymer tailing, and blunt-end ligation. *Escherichia coli* has been exclusively used as the host cells for cloning. However, other microorganisms, eg, *Bacillus subtilis* and *Saccharomyces cerevisiae*, have also been used successfully (104,105). Introduction of the recombinant vectors into host cells (transformation) can be accomplished by different methods, depending on the vector–host cell system used. A calcium heat-shock treatment has been used exclusively in the plasmid–*E. coli* system (106). Successfully transformed host cells can be selected from the whole population using the drug resistance and nutritional markers carried by plasmid vectors, the plaque-forming abilities of phage vectors, immunochemical methods by means of antibodies directed to the substance of interest, or nucleic acid hybridization methods (107,108). Extensive reviews on the application of genetic engineering in vaccinology are available (109,110).

The recombinant hepatitis B vaccine is the first approved human vaccine based on a genetic engineering technique. The viral antigen is produced by a yeast expression system. Almost all new protein antigens being developed such as HIV-1, herpes, rotavirus, and malaria are derived from genetic engineering techniques. The genetic engineering techniques can also be used to reduce the virulence of a pathogen that can then be used to produce vaccines. Thus, a mutant of pertussis has been constructed that will produce a cross-reactive material of the pertussis toxin (111). It has none of the toxicity associated with pertussis toxin, but it still produces the immunological properties of the antigen. This vaccine has been shown to be effective and is approved for use in several countries. A similar technique is being used for developing other vaccine candidates (112). Vaccine candidates for *Salmonella typhi* have been generated (112,113) by deleting the genes in the aromatic amino acid pathway. This vaccine candidate can be used to protect against typhoid. Similar vaccines are being developed for shigella and cholera (112). Live vectors (114) are another application of genetic engineering. In this case, the genes from a pathogen are inserted into a vaccine vector, such as salmonella or vaccinia. In the case of salmonella, it will be possible to develop an oral vaccine. Vectors for this application include salmonella, BCG, polio, adenovirus, and vaccinia.

The use of naked DNA as a vaccine is the most recent development in this field. Since the demonstration of the possibility of genetic immune response by direct injection of DNA into muscle cells, the field is developing rapidly (115).

Not only does it allow for large-scale production of vaccine, the use of naked DNA also has the advantage of stimulating the desirable Th 1 response (116). The main obstacle for this approach is the low uptake rate of the injected DNA. Thus, the recent developments in this field are the different approaches in preparing DNA and injection techniques for improving the immune response (117). Clinical trials for influenza (118), hepatitis (119), HIV-1, and herpes simplex are being initiated.

9. Adjuvants

Adjuvants are substances that can modify the immune response of an antigen (120,121). With better understanding of the functions of different arms of the immune system, it is possible to explore the effects of an adjuvant, such that the protective efficacy of a vaccine can be improved. Recent vaccines are highly purified antigens and have the advantage of increased safety. However, they suffer from decreased immunogenicity as compared with the killed pathogen vaccines. Fortunately this can be addressed by the use of an appropriate adjuvant.

A vaccine adjuvant can have several influences on immune response. The properites of vaccine adjuvants include enhancing the kinetics and presistence of response to antigens, increasing not only antibody quantity but also avidity, specificity, isotype, and subclass. Another influence of adjuvants is enhancement of cytotoxic T lymphocyte response. This property can be especially important in the development of recombinant antigen vaccines for viral or intracellular organisms. Adjuvants can also increase immune response in immunologically compromised individuals, including immunologically immature (neonates), aged, and immune suppressed individuals. Adjuvants can also decrease the amount of antigen required to invoke a protective response, thus potentially reducing other undesirable or unrelated side effects, resulting in high doses, reducing the cost of the vaccine, and/or improving the ability for rapid response to the vaccine crisis with low antigen supply. Finally, some adjuvants aid muscosal immune response (122).

Several mechanisms have been proposed for the action of the adjuvants. Some adjuvants can be considered antigen delivery systems that influence immunogenicity through antigen retention. These adjuvants form a “depot” from which antigen can be slowly released *in vivo*, thus ensuring a prolonged exposure to immune cells. Another method by which these antigen delivery systems may enhance antigen presentation is by enabling increased uptake by phagocytic cells.

Currently, aluminum salt is the only adjuvant approved for use in human vaccines. New adjuvants such as QS-21 (123), Peptidoglycan (124), GM-CSF (125), heat shock protein (126), and others are being evaluated. Several of these adjuvants have been in clinical trial, but none have been approved for human use. IL-12 has been proposed as an adjuvant that can specifically promote T-helper 1 cell response and can be a very promising adjuvant for future vaccine development (127).

10. Peptide Vaccines

Development of a peptide vaccine is derived from the identification of the immunodominant epitope of an antigen (128). A polypeptide based on the amino acid

sequence of the epitope can then be synthesized. Preparation of a peptide vaccine has the advantage of allowing for large-scale production of a vaccine at relatively low cost. It also allows for selecting the appropriate T- or B-cell epitopes to be included in the vaccine, which may be advantageous in some cases. Several vaccines based on peptide approaches, such as SPf66 (76) for malaria and an HIV-1 peptide (129) and polypeptide conjugate for Alzheimer's disease (130), have been in clinical trials. No peptide vaccines are licensed as yet.

11. Combination Vaccines

Breakthroughs in immunology, molecular biology, biochemistry, and other related fields along with a greater understanding of pathogenesis have resulted in many new vaccines as well as improvements in several existing vaccines. Combination vaccines (defined as two or more vaccines in a physically mixed preparation) provide a way in which to encourage increased use of a wide range of vaccines in a broad population. The combination vaccine is usually mixed at the time of manufacture, but it also may be mixed immediately before being given to the subject.

With the introduction of newer vaccines, what has started as five injections in the first two years of life has become as high as 15, leading parents and health-care practitioners to become concerned with the number of needle punctures. This plethora of vaccines creates increased needs for recordkeeping and storage that could discourage health-care providers from administering the full range of indicated vaccines. These issues have stimulated major efforts toward the development of multidisease combination vaccines that could reduce the number of injections in children (131).

The concept of combination vaccines has been developed and exploited throughout most of the twentieth century. DTP vaccines have been available as multidisease combinations for about half a century, whereas pneumococcal, influenza, and polio vaccines have been available as multivalent combinations for several decades. Combination vaccines can be both a live (eg, MMR, polio) or an inactivated/nonlive/killed subunit (DTP, HB, Hib). There are many challenges in the development of new combination vaccines, including technical, clinical, regulatory, manufacturing, marketing, and clinical-use issues. The key to the successful development of combination vaccines lies in identifying all such issues for each proposed combination and in developing a comprehensive integrated plan that addresses these challenges toward the beginning of the program. A key challenge for combination vaccines is that vaccine antigens, which have been developed and licensed separately as vaccines, are brought together into physical mixtures that are not always stable and potent. Thus, pharmaceutical and technical developments of combinations must be performed in a way that an immunogenic, stable, safe mixture in appropriate containers is developed according to accepted regulatory standards (132).

Another challenge with the development of the combination vaccine is that the immunogenicity of a particular component, when given individually, often is not the same as in a combination; when diminished in combination, it is called interference. There are key statistical considerations in determining

the appropriate size of consistency trials for proving that the combination is as immunogenic as its components given individually. As it is not usually possible or ethically acceptable to test the clinical efficacy of a combination vaccine in terms of some or all of its components, it is very useful to define a type of immune response associated with protection, ie, immunological correlate of efficacy (133). Such correlates may be used effectively in place of efficacy for the evaluation and licensure of combinations. The ideal correlate of effficay is one for which there is an assay and a defined quantitative level of antibodies associated with protection.

Regulatory guidelines have been developing for combination vaccines. A vaccine developer needs to anticipate multiple issues during development (134). It is clear that consultation with regulatory agencies early in the development can be very beneficial for assuring that the final formulation and its associated data meet regulatory expectations. From the perspective of the regulatory agency, issues regarding the control of manufacturing and clinical safety and efficacy (immunogenicy) are paramout (135).

12. Other Developments

With the advance of immunology, the scope of vaccinology is also expanding. The technology can be applied into developing vaccines against cancer, allergies, and autoimmune diseases. Rapid progression in these areas has been documented in recent years. With the increased emphasis on bioterrorism, there has been renewed interest in stockpiling of vaccines against smallpox and other rare but highly pathogenic and contagious diseases.

13. Process Technology

In the preparation of classic killed or toxoid vaccines, simple process technology was used. With the advance of new vaccines, far more sophisticated process technologies are needed. The desire to reduce side effects of vaccination requires processes that will yield antigens of extreme purity. The new regulation in cGMP requires consistent production procedures, and global competition also demands that the most efficient process technology be applied.

The basic process technology in vaccine production consists of fermentation of the desired organism for the production of antigen, purification of antigen, and formulation of the final vaccine. In bacterial fermentation, technology is well established. For viral vaccines, the cell culture is the standard procedure. Different variations of cell lines and process systems are in use. For most live viral vaccine and other subunit vaccines, production is by direct infection of a cell substrate with the virus.

Alternatively, some subunit viral vaccines can be generated by recombinat DNA techniques and expressed in a continuous mammalian cell line or insect cell line. Recent advances in bioreactor design and operation have improved the successful production of IPV in large-scale bioreactors. However, roller bottles or flasks are still used for most current vaccine production. Development of an

insect cell culture will allow for a very large-scale liquid suspension culture (136). Several vaccine candidates such as gp160 for HIV, L1 for HPV, and gD protein for herpes have been demonstrated in the insect cell culture and recombinant baculovirus vector system. However, no vaccine has yet been approved for human use.

The purification of an antigen normally uses the most advanced technology available, due to the high value of the product. The antigens are mostly protein or polysaccharide, which can degrade easily. To avoid the degradation of these products, more drastic purification procedures cannot be used. The most common separation procedures are ultrafiltration and chromatography. The chromatography methods are ion exchange, gel permeation, and hydroxyapatite. Affinity chromatography can also be used, but it requires extensive regulatory reviews. Classic separation procedures such as salting out or solvent precipitation are also used.

Development of conjugate and peptide vaccines requires the typical organic synthesis process and purification. This is a new area for vaccine technologists. Again, the main concern is to maintain the immunogenicity of the vaccine candidate during the chemical reaction and purification steps. Most of these procedures are proprietary. Formulation development is also becoming more complex for preparation and delivery of new vaccines. The classic vaccines are mostly prepared as injectable solutions. Aseptic techniques are required in the design and operation of the facilities.

To take advantage of the advance in immunology and adjuvants, future vaccines will be formulated to target a specific part of the immune system. The desire of combining several antigens to reduce the number of injections will require a detailed study of the vaccine formulation. Oral and intranasal delivery may also become common practice. All of these will need different technology for the preparation of the final vaccine dosage form and will present new challenges in vaccine technology.

14. Economic Aspects

An important aspect of vaccine technology is the cost–benefit relationship between prevention vaccination and disease treatment. Generally the cost savings are high. For the early period of polio immunization (1955–1961), the net savings as a result of immunization were calculated to be $327 million. If loss of income was added, the savings would amount to ~$1 billion. Measles vaccination was estimated to have saved $100 million in medical and lost work costs from 1963 to 1967 (137). Studies of the cost effectiveness of immunization of children against diphtheria, tetanus, and pertussis disease have yielded a benefit-to-cost ratio of 6.2:1 for direct costs and 20.1:1 when indirect costs are included (138,139). Projected savings from a rotavirus immunization program have also been calculated. A partially protective vaccine would yield an average savings of $78 million per year in the United States in health-care costs and $466 million when overall costs to society were considered (140). Direct and indirect savings for commonly used childhood vaccines as studied by the CDC are given in Table 3.

Cost–benefit analyses for adult immunizations have also been performed. Influenza immunization during the period from 1971 to 1977 resulted in over 13 million more years of life at a cost of only $63 per year of life gained. Productivity

Table 3. **Benefit–Cost Analysis of Commonly Used Vaccines**[a]

Vaccine	Direct medical savings, $	Direct and indirect savings,[b] $
DTP	6.0	29.1
MMR	16.3	21.3
OPV	3.4	6.1
Integrated schedule (DTP, MMR, OPV combined)	7.4	25.5
H. influenzae type b	1.4	2.2
Varicella	0.9	5.4

[a]Savings per dollar invested.
[b]Indirect savings include work loss, death, and disability.

gains were estimated to have a value of $250 million (141). Projected costs of pneumonia have been calculated at 3.6 times the cost of vaccination, or a savings of $141 per person is achieved among those at risk for developing pneumonia over the age of 50 years (142).

The worldwide market is approximately $10 billion in sales in 2005 based on individual company earnings. The majority of vaccines for the U.S. market are produced by Glaxo SmithKline, Merck, Sanofi Pasteur, and Wyeth. Costs of vaccine manufacture vary according to the type of vaccine produced and how it is supplied. Live virus vaccines are generally less expensive because the quantitative mass to be given to the recipient is less than an inactivated or subunit vaccine. The purification process and yield and the number of strains or components in any given vaccine also affect the cost of manufacture. New vaccines often have a royalty cost, in addition to manufacturing and testing costs. Because of the requirement for specialized facilities and training of personnel, there is a large fixed cost burden to the manufacturing process, making production volumes a key factor in overall cost per dose. Filling and packaging is often the most expensive part of the manufacturing process, and the cost varies by how many doses are filled and packed into one unit.

In addition to covering the cost of goods, the price of vaccines to the consumer must also be able to cover sales, marketing, and distribution costs (including a cold chain, a vaccine shipping and storage temperature requirement), and research and facilities for new products. The setting for immunization will affect the cost to the consumer. Slightly more than half of the vaccines used in the United States for pediatric immunization are purchased in large volumes by the government and administered in the public sector environment, whereas the remainder are administered in the private practice sector. An excise tax on vaccines, used to cover adverse reaction events, is also included in the price. Full immunization in the public sector was estimated at $111 and in the private sector at $238 per child in 1993 (143).

Liability for adverse reaction events associated in time with immunization have also played a principal role in vaccine economics. Prior to 1988, compensation for any adverse reaction associated in time with vaccination required that the vaccine recipient bring suit against the manufacturer or the health-care provider that administered the vaccine. The uncertainty of numbers and costs associated with lawsuits contributed to the decline in the number of providers of

routine childhood vaccines during the late 1970s and 1980s. Lawsuits peaked for the DTP vaccine in 1986, leading to the enactment in 1988 of the National Vaccine Injury Compensation Program. This program, as well as the National Childhood Vaccine Injury Act of 1986, was provided as a nonfault alternative to the tort system for resolving claims resulting from adverse reactions to mandated childhood vaccines, and it has achieved its goal of providing compensation to those injured by rare adverse events associated with vaccination and providing some stability for the vaccine market.

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GOPALAM SOMASEKHAR
SHOU-BAI CHAO
Wyeth Biotech