7. Immunization Technologies: Selected Tropical Diseases

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INTRODUCTION

Vaccines exist for many of the viral and bacterial diseases that are important in developing countries, but there are no vaccines against the parasitic diseases that are generally associated with the tropics. Antiparasite vaccines are more difficult to formulate than vaccines for viruses and bacteria, the reason being that protozoan and helminthic parasites are more complex in biochemical and physical composition, life cycle stages, and interactions with their hosts. The immunology of parasitic diseases is in a vigorous phase of research (66,164,213,241,273) and forms the underpinning for attempts at vaccine development.

The available vaccines vary in the protection against disease that they induce and the risks of adverse effects in individuals who are vaccinated. Vaccines against yellow fever, measles, rubella, and mumps, for example, are almost always effective and provide for long-term protection. Others—influenza, pneumococcal pneumonia, cholera, and typhoid, for instance—do not induce immunity in as high a proportion of those vaccinated and may last only a matter of months or a few years. Both new vaccines against diseases currently not immunizable and improvements in existing vaccines are needed.

There is great optimism in vaccine research today. Large strides in biotechnology, particularly in the use of monoclinal antibodies (MAbs) and recombinant DNA, and a rapidly growing body of knowledge in immunology are aiding the field of immunization technology. The development of replacement vaccines for viral and bacterial diseases that are safer, more effective, and possibly less expensive than vaccines currently available, and the development of new vaccines against some tropical diseases, including parasitic diseases for which no vaccines now exist, are advances that now depend more on research with the tools already at hand than on major technological breakthroughs (310).

The single most visible, exciting development in vaccines today is that a vaccine against malaria may be ready for widespread use in a matter of a few years. In 1984, progress could be followed almost weekly, as the immunologic properties of various life-stages of malaria parasites were characterized in laboratories in different parts of the world and the information was processed for practical significance to a vaccine.

IMMUNITY AND VACCINES: BACKGROUND

Immunity

The term "immunity" refers to the capacity of an organism to resist a particular disease. Disease-causing organisms themselves require certain conditions for survival, and only certain potential host organisms will provide those conditions. Thus, as a species, humans are susceptible to a unique set of pathogens that differs from the set of pathogens that cause disease in other species, although there are areas of overlap (zoonoses). "Species immunity" does not stem from a response of the potential host's immune system against the disease, but simply from an inability of the disease organism to become established. From an evolutionary point of view, such immunity is important, but from the standpoint of preventing diseases and developing vaccines against them,

the important kind of immunity is that which individuals have or can develop against diseases that do occur in human beings.

The immune status of an individual is built from a complex of mechanisms, some genetically determined and some acquired during life, which we are just beginning to understand. A person with complete immunity will not get a disease when exposed to the causative agent. The converse is complete susceptibility, which means that anytime the person comes in contact with the causative organism, the disease will develop. Between solid resistance and complete susceptibility, a range of states of partial immunity and partial susceptibility exist.

"Innate immunity" is genetically determined at birth. Many people of African descent are resistant to *Plasmodium vivax* malaria because they lack a protein on their red blood cell surface that normally allows the malaria parasite to bind to the red blood cell. Many people in endemic malaria regions are resistant to P. *falciparum* malaria because they carry the sickle-cell or thalassemia trait. Genetically determined aberrant types of red blood cells cause sickle-cell anemia in individual who inherit the aberrant trait from both parents (homozygotes), but prevent malarial infection in individuals who have inherited the trait from only one parent (heterozygotes).

"Acquired immunity" is immunity that results either from the body's response to exposure to disease or from vaccination ("active immunity") or from the transfer of antibodies from another person or from animals ("passive immunity"). Active and passive immunity are discussed further below.

Active Immunity

"Active immunity" is immunity acquired by an originally susceptible individual by effective contact with an infectious organism (or a closely related species) or its products. Such immunity results when a biochemical entity known as an antigen (which may be on, in, or produced by the infectious organism) stimulates a response by the immune system of the host.

Active immunity is the result of two general types of stimulation of an individual's immune system:

- **humoral immunity: immunity resulting from the production by antigenically stimulated B-lymphocytes (specialized white blood cells) of nonliving proteins called "antibodies" (or immunoglobulins) that circulate in the blood; and**
- **cell-mediated immunity: immunity resulting from increased activity by living cells in the blood and other tissues (e.g., T-lymphocytes, macrophages/monocy tes, eosinophils, mast cells, and natural killer cells) that directly and nonspecifically destroy foreign material in conjunction with antibodies.**

Active immunity often is a result of natural exposure to an antigen that results in subclinical or clinical infection (e.g., chickenpox infection), but it may be induced by vaccination (e. g., measles or polio vaccination).

Vaccines usually stimulate humoral immunity, i.e., antibodies against a specific antigen, but some immunizing agents strengthen cell-mediated immunity. An example of the latter is Bacillus Calmette-Guerin (BCG) vaccine, a weakened or attenuated tuberculosis vaccine. Whether stimulated by natural infection or vaccination, active immunity usually takes some weeks to develop and may thereafter be lifelong, as with yellow fever or smallpox immunization, or may wane after a variable period, as with typhoid fever.

Passive Immunity

"Passive immunity" is immunity conferred on an individual by the direct transfer to that individual of antibodies produced by another individual. It may result from direct transfer of immune serum that is obtained from individuals with acquired immunity against a specific infectious agent. Large injections of immune serum globulin against hepatitis ("gamma globulin") are sometimes given to Western travelers to the tropics. Newborns acquire certain antibodies passively through the placenta from their mothers and also receive some protection through antibodies in

breast milk. The protection offered by passive immunity is relatively short-lived, usually disappearing within a period of weeks. The transfer of antibodies does nothing to stimulate the body's immune system to produce its own antibodies.

The use of MAbs to confer passive immunity against experimentally attempted infection ("challenge") is important for laboratory investigations and for establishing the theoretical possibility of inducing immunity, but probably will not become a major tool for disease control.

Vaccines

Vaccines are used to produce active immunity. In all cases, a vaccine contains some biochemical compound whose structure is the same as, or similar to, some part or product of the infecting organism. Introducing that foreign material (antigen) stimulates the host's immune system to produce humoral and cell-mediated immunity. Following vaccination, the host's immune system is primed to attack the organism more quickly and efficiently should it ever reinvade the host's body.

Most tropical diseases are chronic infections (e.g., malaria, schistosomiasis, leprosy) in which the proliferation of the pathogen does not exceed a certain level and an equilibrium of sorts is established between pathogen and host. The immune system of the host reacts to the parasite, yet the parasite is not completely eliminated because it has various methods of evading the immune system. The equilibrium reached is "beneficial" to the parasite: the host's body is not overwhelmed or killed by the infection. Parasites evade or subvert the immune system in many different ways. They can lose the surface antigen by which they are recognized by the host, take up host proteins as a surface coat, shed and generate altered surface antigen over time, or internalize in the host's cells, The challenge of current immunology is to make the immune system produce an effective response against the parasite.

A further complexit, is that the immune response itself often causes the pathology of the disease. For example, the immune response to schistosome eggs in the host's tissue causes the clinical symptoms of schistosomiasis. In eliciting a protective response by the host's immune system, it is important to avoid stimulating a response that causes immunopathology.

Types of Vaccines

The production and composition of vaccines differ depending on the disease-producing organism and the available knowledge about it. It is no accident that most currently available vaccines are for diseases caused by viruses and bacteria, which have limited variability in their structure, biochemistry, and genetic makeup. Most of these vaccines were created through empirical work, often with very imperfect understanding of the underlying biochemistry and molecular biology.

Conventional Vaccines.—The commonly produced vaccines are derived from actual disease organisms (or closely related organisms) grown in a culture medium, embryonated eggs, or in cell culture. Despite great efforts at quality control, such biological products are always subject to some variation. Potency can vary, improper inactivation may make a virulent vaccine, and organisms may change or mutate in culture, reverting to or developing pathogenicity. Another potential problem is contamination. For example, some early batches of polio vaccine grown on monkey kidney cell cultures were contaminated by a monkey virus. In some vaccines, there maybe latent, undetected viruses whose effects may not be evident for years.

The major types of conventional vaccines are the following.

1. **Live infectious organisms:** The infectious agent is obtained from someone who has the disease. For instance, scrapings from lesions of cutaneous Ieishmaniasis have long been used in endemic areas for deliberate inoculation of children to prevent the possible appearance of disfiguring facial lesions later in life (168).

2. **Closely related organisms that stimulate cross-immunity:** Jenner's discovery in the 18th century that inoculation with cowpox could prevent smallpox marked the beginning of modern vaccination.

3. **Live, attenuated organisms: Cultured organisms are weakened in various ways so that they**

will remain infectious and stimulate immunity but will not cause disease. Polio, measles, rubella, and BCG vaccines are of this type. Among the problems with this approach are the lack of assurance that the attenuation procedures really eliminate pathogenicity while maintaining appropriate antigenicity; the possibility of mutational reversion to virulence; the introduction of pathogen genomic segments into host DNA; and a small but measurable incidence of severe side effects such as encephalitis. The antiparasite vaccines in use in animals are live, attenuated organisms, the most successful of which are the radiation-attenuated bovine lung-worm larvae employed in some countries since 1959 (26). Field trials have also been reported with irradiated larval schistosomes in cattle (338).

4. Killed (inactivated) organisms: By heat, formalin, or other methods, the disease-producing organism is inactivated without destroying its immunogenic potential. Typhoid, pertussis, plague, and Rocky Mountain spotted fever vaccines are examples of vaccines produced in this way.

s. **Products of bacteria: When a specific biochemical product of bacteria produces the disease, this toxin may be altered to a nonpathogenic toxoid, which can be used to immunize. Tetanus and diphtheria vaccines are toxoids.**

6. Parts of organisms (subunit vacanes): The purified pneumococcal or meningococcal' capsule polysaccharide subunit vaccines are prepared by isolating an immunogenic portion of the outer surface of the pneumococcal or meningococcal bacterium. Isolated polysaccharides are only weakly immunogenic, especially in children. Efforts to increase immunogenicity by coupling polysaccharides to carrier proteins are in progress. The term "subunit vaccine" also is used to describe vaccines made from subunits of bacterial toxins (e.g., the subunit-b vaccine for cholera).

Synthetic Subunit Vaccines.—Much current work is directed toward the development of vaccines consisting of uniquely specific antigenic molecules of completely known structure. It is hoped that such materials, of uniform high quality, can **be** synthesized in production volumes, providing inexpensive, safer, and more effective products.

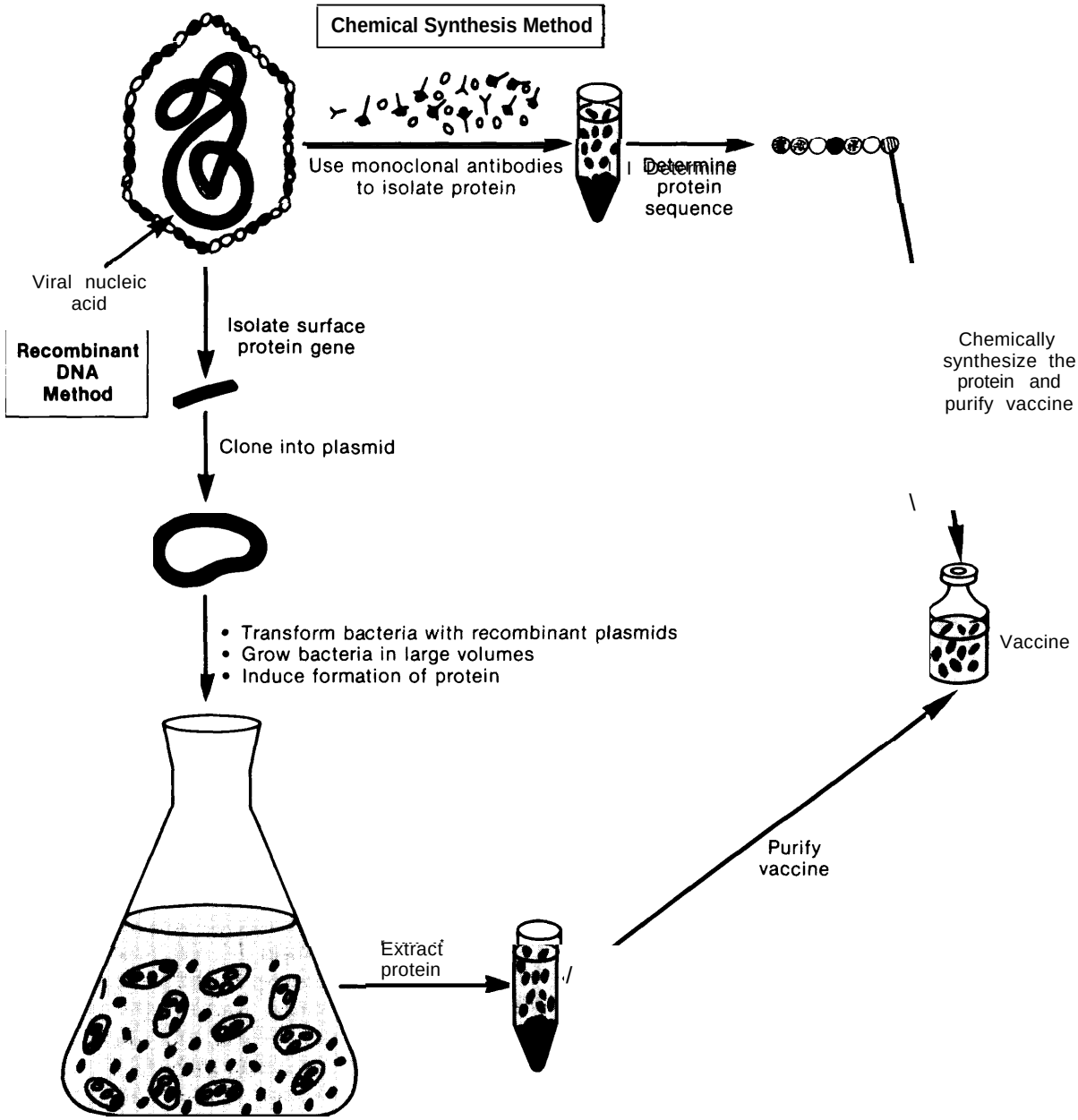
1. **Recombinant DNA products: Three strategies using recombinant DNA technology are useful in synthesizing vaccines:**

a. Antigenic substances that stimulate protective immunity are identified or extracted from the pathogenic organism. Appropriate segments of pathogen DNA, coding for production of those antigens, are spliced into the DNA of another organism. In some cases, the host organism is a yeast or bacterium which thereafter synthesizes the desired molecule. The protective antigen is then separated from other materials in the culture and prepared as a vaccine (see fig. 7-l).
b. The host organism (from a above), if nonpathogenic, can be used to infect the vaccinee, who then produces an immune response against the harmless carrier organism and the "piggy-backed" antigen. The nonpathogenic carrier organism can be either a related strain of the disease-producing DNA donor or an unrelated species.
c. A pathogenic organism can be "attenuated" by deleting the specific gene causing disease, thereby rendering the organism nonpathogenic but still antigenic.

2. **Total synthetic antigens: If a specific, defined protein or polypeptide (amino acid chain) is determined to induce protective immunity,** scientists may be able to produce this antigen in pure form by a total chemical synthesis (see fig. 7-1) and use it to induce an immune response (314).

3. Anti-idiotype vaccines: In order to make anti-idiotype vaccines, scientists produce a passively protective MAb against the disease organism, then produce a second MAb against the first MAb. The second MAb (in effect, an "anti-antibody") may then be used as an "antigen" to stimulate production of the original protective antibody in a host. This application of MAbs would be most significant where an immunogenic protective antigen is in short supply and not amenable to synthesis by recombinant DNA or chemical techniques.

Figure 7-1.—Methods Used To Prepare Subunit Vaccines Against Viral Diseases: The Recombinant DNA Method and Chemical Synthesis Method



In the chemical synthesis method, proteins that comprise the viral surface are isolated, often with the use of monoclinal antibodies (MAbs). The protein sequence is then determined. Based on the sequencing information, large amounts of the protein or portions of the protein are made chemically for use as the vaccine.

In the recombinant DNA method, the gene that encodes the viral surface protein is isolated and cloned into an appropriate vector (e.g., plasm id), transformed into a host (e.g., a bacterium or yeast), and the host is grown in large quantities. Formation of the protein by the recombinant DNA and isolation of the protein results in the subunit vaccine.

SOURCE: Adapted from Office of Technology Assessment, *Commercial Biotechnology: An International Analysis,* **1984**

Culture of Disease-Producing Organisms

Major steps toward producing vaccines against tropical diseases have been made possible by recent advances in the culture of disease-producing organisms. A method for in vitro cultivation of *P. falciparum* (343), for example, removed a major roadblock to further research in malaria, namely, the requirement for maintaining this organism in animal hosts. Other advances include success in cultivating in vitro the complete life cycle of *Trypanosoma brucei,* which causes African sleeping sickness (156), and the discovery that armadillos are an excellent culture medium for *Mycobacterium leprae,* which causes leprosy. These technical advances have increased the availability of experimental material for research use, but further advances are still needed.

VACCINATION AND VACCINE RESEARCH: CURRENT STATUS FOR SELECTED TROPICAL DISEASES

Malaria

The development of a vaccine against malaria is one of the great challenges that is now being addressed. Steady progress over the past decade has encouraged the scientific community, and hopes for an effective vaccine within the next decade have been voiced. A detailed account of malaria vaccine development to the present appears in *Case Study B: The Development of a Malaria Vaccine.*

Human malaria is caused by four species of protozoan parasites of the genus *Plasmodium: P. falciparum, P. vivax, P. ovale, and P. malariae.* Other species of *Plasmodiurn* are infective to a wide variety of vertebrates other than humans (e.g., *P. berghei, P. yoeli, and P. chabaudi* to rats and mice, and *P. knowlesi* to rhesus monkeys).

Malaria parasites *(Plasmodium* spp.) have a complex life cycle (see ch. 4) with several distinct life-stages that could provide points of intervention for vaccines. One of the primary targets of current malaria vaccine research efforts is the sporozoite life-stage. Plasmodial sporozoites are introduced into a vertebrate host's bloodstream during the bite of an infected *Anopheles* mosquito. Once in the host's bloodstream, sporozoites move within a short time to infect the liver cells, initiating malaria infection. For a sporozoite vaccine to be effective in preventing malaria in humans, every sporozoite would have to be killed.

Another target of malaria vaccine research are the erythrocytic or blood stages-either schizonts within red blood cells or free merozoites—of the malaria parasite. Merozoites, the stage most directly responsible for the symptoms of human malaria, develop from sporozoites in the infected host's liver cells (exoerythrocytic cycle), burst out of the liver cells, enter the host's circulatory system, and invade the person's red blood cells (erythrocytes). There they undergo asexual reproduction, forming schizonts, which release more merozoites to infect more red blood cells (erythrocytic cycle). In chronic infections, this cycle may be repeated many times. Merozoites are the main target of blood stage vaccine research.

A third target of malaria vaccine research is the gamete, the sexual stage of the parasite. Gametes mature in the mosquito from gametocytes that are picked up when the mosquito bites an infected person. If the person had been vaccinated against the gamete stage, the mosquito would also pick up antibodies circulating in the host's blood formed against the gamete. In the mosquito, the human antibodies would prevent the gametes from completing their life cycle, and thus prevent the mosquito from spreading malaria. Since the gamete vaccine would not protect the vaccinee from acquiring malaria, this type of vaccine is called an "altruistic" vaccine.

Two other potential vaccination targets are the infected liver cells and the infected red blood cells, whose surface membranes may be altered by the presence of parasites in the cell.

Most evidence indicates immunity is stage-specific in malaria. A vaccine against merozoites re-

leased from red blood cells, therefore, will not control exoerythrocytic merozoites released from liver cells (291). Successful immunization in human populations will probably require a combined vaccine against several stage-specific determinants, although a monovaccine against the sporozoite stage may have value to short-term visitors to areas where malaria is endemic.

Early Attempts at Immunization

Experimental malaria immunization was achieved using sporozoite, merozoite, and gametocyte vaccines during the late 1960s to mid-1970s.

Sporozoite Stage.—Immunization with radiation-attenuated sporozoites was shown to give excellent protection in rodents, birds, and human volunteers (19,61,62,261,262). One problem with this sporozoite vaccine is the difficulty of obtaining enough antigenic material in a sufficiently purified form to immunize even selected populations.

Erythrocyte Stage. —Vaccination with merozoites was achieved in rodents, birds, and nonhuman primate models using crude whole parasite antigens or partially purified antigens, but the use of adjuvants (nonspecific stimulators that enhance the immune response) was required (65,67, 68,241,242). (Because of side effects, especially tissue death at the injection site, at least some adjuvants cannot be used in humans.) Vaccination of rhesus monkeys with merozoite preparations of P. knowlesi in Freund's complete adjuvant conferred complete protection against an otherwise fatal infection. Immunity to blood stage parasites was also induced in *Aotus* monkeys with *P. falciparum* and *P. chabaudi* in mice. Various antigen preparations were used in these experiments: whole blood-stage P. *falciparum* parasites with adjuvant; merozoites obtained by "natural release" methods; and glutaraldehyde-fixed and freeze-thawed merozoites. Purified antigens of *P. yoeli* merozoites induced protection in mice. Rhesus monkeys were also vaccinated with an immunogenic protein isolated from the membrane of *P.* knowlesi-infected red blood cells.

Gamete Stage.—Gamete vaccination was demonstrated in chickens and rhesus monkeys. The mechanism of action is transfer (when the mosquito feeds) of antigamete antibodies produced in the animal host which act on the gametes in the mosquito gut (46,142).

Cultivation of *Plasmodium*

Although the possibility of protective immunization against malaria had been established by the mid-1970s, major progress towards producing vaccines required advances in the culture of malaria parasites.

In 1976, Trager and Jensen (343,344) described a method for in vitro cultivation of P. *falciparum* blood stages that removed a major roadblock to further research in malaria. Previously, malaria research had depended on maintenance of the parasite in animal hosts. This situation was unsatisfactory for several reasons: 1) it is expensive to keep animals; 2) large-scale production and isolation of the parasite is usually difficult; 3) biological phenomena of the host affect the malaria parasite and contaminate and increase the variability of any preparations.

In vitro cultivation of *P. falciparum* has become standard laboratory procedure to provide researchers with sufficient parasite material to perform other laboratory research on malaria. Because research on *P. falciparum* is most urgent (because of fatal consequences in *P. falciparum* infection), it was fortuitous that cultivation of *P. falciparum* erythrocytic stages proved easier than other malaria species. Success in cultivating merozoites of the other human malaria species is still elusive.

The next stage of the malaria parasite to be cultivated in vitro was the gametocyte, which when fed to mosquitoes through artificial membranes, led to the development of infective sporozoites (42). This was important for subsequent work on the sporozoite antigen because it ensured adequate amounts of material for study.

Liver stages of the parasite are now also being cultivated in vitro. The complete exoerythrocytic cycle of *P. berghei* (a mouse malaria) has been cultivated in the laboratory (158) (in a cell line of human embryonic lung cells). Attempts to cultivate the exoerythrocytic stages of monkey and human malaria are in progress. The tissue culture system *P. berghei* provides an in vitro assay to test for protective antibodies. It also provides a

model for study of possible chemotherapeutic compounds.

Recent Research

Sporozoite Vaccine Research.—Using the *P. berghei* mouse malaria model and the in vitro tissue culture system, scientists have made important progress in the study of protective sporozoite antigens (63,422). The surface of the mature sporozoite is covered by a major species- and stage-specific membrane protein, the "circumsporozoite protein, " which for *P. berghez"* **is** named Pb44 for its molecular weight of 44,000. Synthesis of the circumsporozoite protein is a major metabolic activity of the mature sporozoite (immature forms of the sporozoite isolated from the mosquito midgut do not have this circumsporozoite protein, nor do later erythrocytic stages).

A very small quantity of purified MAb to Pb44 protects mice against sporozoite challenge (passive immunity). If sporozoites are incubated in vitro with the complete MAb or the antibody-specific portion of the MAb known as the "Fab" fragment, they lose their ability to infect liver cells (279). The antibody prevents attachment to, and penetration of, the mammalian target cells by *P. berghei* sporozoites in vitro (422). Thus, immunity to sporozoites in mice depends on binding of antibody to the malaria surface protein, preventing interaction with target cells.

Sporozoites infect liver cells by first attaching to a receptor on the cell membrane and then penetrating by means of a process involving movement between parasite and host cell (3). Attachment requires a protein receptor, and penetration requires movement of host cell components. Irradiated sporozoites attach, enter, and transform to the next liver stage (trophozoites), but do not develop to the mature liver (schizont) stage.

MAbs, which permit the recognition and isolation of individual antigens in pure form, have identified protective antigens from several species of *Plasmodium (P. knowlesi, P. chabaudi, P. falciparum, P. vivax),* including polypeptides analogous to Pb44 (64,89,431).

Building from the availability of sufficient antigen and a suitable model, scientists have now applied recombinant DNA technology to the production of pure protective sporozoite antigens; biosynthesis of the antigen by expression in Es-*cherichia coli* **of** the gene coding for protective sporozoite surface antigen has been achieved (109,432). The immunogenic region of the antigen has been identified and chemically synthesized (134,434). This work has been reproduced with the *P. falciparum* gene. Recently, the antigen produced by this process has been shown to be protective against *P. falciparum* challenge.

Erythrocyte Vaccine Research.—Erythrocyte antigens are being studied in rodent and primate malaria models and in *P. falciparum.* This research has been successful in producing scientific knowledge, but the implications for a blood stage vaccine are not yet clear.

Species- and stage-specific antigens have been identified on the surface of malaria-infected red blood cells *(P. chabaudi, P. knowlesi, P. falciparum,* and MAbs to at least one antigen showed protective activity *(P. chabaudi).* Membrane antigens of *P. knowlesi* and *P. falciparum* have been compared; clinical immunity to *P. knowlesi* correlated with the presence of antibody to a particular molecule (MW 74,000), which was then isolated and used to immunize monkeys. Antibody to this protein inhibited in vitro multiplication. Thus, there is encouraging evidence that a parasite-derived antigen(s) is at the erythrocyte surface, and it stimulates protective immunity. Recombinant DNA technology is being applied in several laboratories to produce protective erythrocyte stage antigens (16).

However, antigenic variation has been shown in *P. falciparum* in *Aotus* monkeys (159,160). Strain-specific determinants on the surface of red blood cells infected with late-stage parasites were recognized by immune serum. These determinants varied according to host factors. Variant antigens can be identified by surface immunofluorescence, and protection is correlated with the presence of antibody to surface determinants. MAbs against geographic isolates of *P. falciparum* **can** distinguish between isolates, and some can block merozoite invasion of red blood cells.

These findings imply that functionally important antigens, which appear to protect by induc-

ing antibodies, do exist in the erythrocytic stages, but differ between *P. falciparum* isolates, and that *P. falciparum* has the ability to vary surface antigens in response to the body's immune response. The possibility of developing a protective immunization against the merozoite stage appears to be rather poor, if the antigen that stimulates protective antibodies varies not only over place, but also over time. On the other hand, if there is one or more determinant common to all or most strains of the parasite, in spite of the variability, then a vaccine may be feasible.

One fact that may be exploited for a merozoite vaccine is that merozoites recognize receptor "glycoprotein" molecules on red blood cells. These glycoproteins play an indirect role in the invasion of red blood cells.

Other Immunologic Research. -Mechanisms of immunity are being investigated, and greater understanding of cell-mediated immunity (T-cells, macrophages, natural killer cells) has been achieved. Generalized microphage activation, induced by substances unrelated to malaria, provides some protection to mice from *P. berghei* infection, perhaps by increasing phagocytosis of parasitized red blood cells (334).

Researchers at the National Institutes of Health (NIH) have demonstrated that *P. falciparwn*-infected red blood cells attach to the cells that line blood vessels (endothelial cells) and to cells of a particular melanoma (skin cancer) cell line. This is confirmation of the longstanding hypothesis that *P. falciparum* somehow differs from the other species by being "sticky" and thus adheres to and clogs the blood vessels. This in vitro model allows further investigation of this phenomenon, especially in relation to the pathogenesis of cerebral malaria. The implication for vaccine research is that somehow the surface of malaria-infected red blood cells are different from normal red blood cells, a difference which perhaps can be exploited to selectively eliminate these cells.

Tropical splenomegaly syndrome (TSS), a complication of malaria infection, found especially in children in endemic areas, is being studied, and evidence obtained indicates that antibody responses in TSS are specific to malaria. Since TSS may be some type of immune disease brought on by the body's response to infection, it must be considered and evaluated in the development of a vaccine.

The immunosuppressive effect of extracts of malaria parasites is being investigated with the *P. berghei* model. Certain components of malaria parasite chemical extracts have been shown to suppress T-cell-dependent immune responses to other pathogens. This finding may help to explain why malaria infection lowers the body's immunity to other infections and reduces the immune response to immunization for other diseases (e.g., measles vaccine). Further research in this area is needed.

Schistosomiasis

Schistosomiasis affects about 200 million people in parts of Africa, the Caribbean and South America, and the Orient. The major schistosomes infecting humans *(Schistosoma mansoni, S. japonicum,* and S. *haematobium)* have life cycles requiring certain freshwater snails as intermediate hosts.

Immunologic Research

Studies of schistosome infection using immunologic techniques have provided much new information of relevance to immunology itself, such as the first knowledge about the biological function of eosinophils (a type of white blood cell) and one of the immunoglobulins. In fact, it is only a slight overstatement to say that schistosomiasis has done more for immunology than vice versa.

The eosinophil is one of several types of white blood cells involved in cell-mediated immunity. The schistosome model is the most extensively studied laboratory system with respect to eosinophil involvement in immunity. There is mounting evidence that acquired resistance in schistosomiasis involves an antibody-dependent (humoral) mechanism that somehow activates the eosinophil to kill the foreign organism (19). The schistosomulae (the earliest life-stage of the schistosome in the body of the human host) appears to be open to attack by two mechanisms: 1) schistosomulae are coated with IgG antibody and then are attacked by eosinophils (39); and 2) IgE antibody-coated

schistosomulae are attacked by activated macrophages, specialized cells of the immune system (44).

In addition to functioning at sites of skin penetration by schistosome larvae, eosinophils predominate in granulomatous lesions which develop around schistosome eggs entrapped in host tissues. The egg granuloma has been shown to be a principal factor in the pathogenesis of liver and spleen involvement in schistosomiasis, and histologic studies have indicated that the eosinophils destroy schistosome eggs. If eosinophils are eliminated by administration of anti-eosinophil serum, there is marked reduction in size of egg granulomas and concomitant decrease in pathology. This is an example of pathological damage to the human host arising from the host's own immune function.

Many laboratories have produced antischistosomal MAbs for identification of relevant antigens or for species identification (e.g., 83,84,99, 234,236,332,336). Dissous and colleagues (96) have reported protection against S. Mansoni challenge in rats achieved by passive transfer of a rat antischistosomal MAb. This MAb recognized an antigen on the surface of immature worms and a different antigen on adult worms, both antigens sharing a common epitope. Other investigators have also reported passive protection against S. rnansoni challenge, using monoclonals in mice (320,436).

Current Status

A live irradiated larval vaccine for schistosomiasis has been administered to cattle in the Sudan with encouraging results (338), but it is not acceptable for use in humans (because the larvae must infect the host, even though they do not mature to egg-producing adults).

Prospects for a subunit vaccine for schistosomiasis are not clear. Immunologic research has demonstrated the complexity of the immune response to the parasite and the parasite's protective responses. Developing schistosomes acquire host antigen on their surface, which masks them from the host immune response (130). The adult worm is largely protected from immune attack by acquisition of host antigen, shedding of antigen, and/or antigenic variation. The adult schistosome worm can survive for many years, apparently immune to host attack, yet stimulating immunity against further infection by larval schistosomes, thus becoming a chronic infection. Most evidence points to the early schistosomulae (the first immature infective stage) as the most likely point of attack for a vaccine.

Trypanosomiasis

Trypanosomiasis is a group of clinically differing diseases of the blood and tissues caused by several species of the genus Trypanosozna. The important human diseases are: African sleeping sickness (African trypanosomiasis), an acute form caused by T. *brucei rhodesiense,* and a chronic form caused by T. *b. gambiense;* and Chagas' disease (American trypanosomiasis) caused by T. *cruzi.*

African Sleeping Sickness (African Trypanosomiasis)

Current Status.—Currently, there are no vaccines against African sleeping sickness. One of the larger research efforts in vaccination for African trypanosomiasis is being carried out at the International Laboratory for Research on Animal Diseases (ILRAD) in Nairobi, Kenya, in an effort to prevent a similar disease in cattle (nagana) that has a devastating economic impact. The Special Program for Research and Training in Tropical Diseases (TDR) has a very modest effort that defers to ILRAD's effort.

Recent Progress.—In the past few years, scientists have made significant advances in demonstrating genetic mechanisms of antigen switching in African trypanosomes, with corresponding regulation of the expression of variant surface glycoprotein (VSG) genes. This work is important to vaccine development because surface proteins are the most likely antigens to stimulate the immune system.

Research on VSGS is now being pursued intensively, and it appears that at least two distinct mechanisms operate to cause shifts in VSGS: 1) changes in the gene itself, including duplication of a preexisting invariable basic copy of the VSG gene, followed by transposition of the newly duplicated "expression-linked copy" to an area of

the genome in which it can be expressed; and 2) changes in gene activation related to a small segment spliced on to one end of the corresponding messenger RNA. Several types of genomic rearrangements have been described (22). Complementary DNA (cDNA) has been prepared for a variety of VSGS. Scientists have found that the VSGS are encoded by a fairly large number, perhaps up to 1,000, separate genes. DNA sequencing has been done on several VSG genes, which show considerable similarities over a large segment (290). The complete nucleotide sequence of cDNA coding for a VSG of T. *brucei* has been worked out by Boothroyd and colleagues (25), and the manner in which the VSG is made from more complex precursors is becoming known.

Current work on African trypanosomes by American investigators is centered largely on the molecular biology of antigen switching of VSGS. Roughly half a dozen laboratories are working intensively on mechanisms of transcription and expression, the nature of requisite messenger RNA splicing, evolution of the VSG repertoire, and similar problems. Although some workers are attempting to characterize the complete antigenic repertoire of African trypanosomes and are searching for nonvariant antigens across strains, there is little hope of developing a vaccine in the near term. The hope is that this research will pay off in the short term by identifying discriminatory proteins for use in immunodiagnosis and metabolic targets for chemotherapy.

Chagas' Disease (American Trypanosomiasis)

Current Status.—The current status of vaccination for Chagas' disease is generally considered unpromising. Experimental vaccines have been made by conventional methods (339) but have not been widely adopted. T. *cruzi* appears to be a good candidate for application of MAbs to develop vaccines. Immunization against T. *cruzi* is complicated, however, by the possibility that the severe immunopathologic events of Chagas' disease may be exacerbated by a vaccine.

Wood and colleagues (410) have reported production of a MAb against rat nerve tissue that cross-reacts with T. *cruzi.* Thus, a trypanosome antigen, which is identical to, or close to, a protein that is in host tissue, may be important in the pathology of this infection. It maybe that the host immune system is stimulated to produce antibody against the parasite, that coincidentally cross-reacts with host tissue in a type of autoimmune response. The implication is that a vaccine that stimulates a protective antibody may stimulate immunity and pathology at the same time.

Recent Progress.—MAbs to culture and intracellular stages of T. *cruzi* have been made by several workers. Araujo and colleagues (10) reported production of 17 different secreting hybridomas, of which 5 recognized the antigens of intracellular stages only, 2 recognized *culture* forms only, and 10 reacted with both. Immunofluorescence procedures pinpointed the specific antigenic sites on the T. *cruzi* organisms. Snary and colleagues reported production of MAbs recognizing different stages, but not different species of trypanosomes (323), demonstrating the presence of stage-specific determinants.

In a remarkably original experiment, Crane and Dvorak (80) reported the fusion of T. *cruzi* organisms directly with vertebrate cells using a procedure analogous to hybridoma cell fusion. Three hybrid clones continued to express T. cruzi antigens for at least 14 weeks of serial cultivation, suggesting the continued presence of functional parasite DNA. A great deal of research activity with MAbs is under way. The research is aimed at identifying protective antigens which may be suitable for use in a vaccine, but progress is still elusive.

An animal model for Chagas' disease that mimics the human disease is being pursued by several laboratories. The objective is to have an experimental system for evaluating immunologic responses to T. *cruzi* and the protective effect of any candidate vaccines.

Research Needs.—A vaccine against Chagas' disease is badly needed, but researchers are not highly optimistic that one can be developed (16). In lieu of a vaccine, one investigator demonstrated some protection in mice by passive immunization with a MAb, suggesting that a different immunologic approach to parasite reduction maybe feasible (16). Improvements in drug therapy for

Chagas' disease, rather than vaccination, maybe a more urgent and feasible priority.

Leishmaniasis

Leishmaniasis is caused by several species of the protozoan genus Leishrnarzia. Differefit species cause different clinical diseases:

1. cutaneous Ieishmaniasis;
2. mucocutaneous leishmaniasis, which begins as a skin lesion that progresses to destruction of tissue and cartilage of the face, nose, and throat; and
3. a visceral form of leishmaniasis known as "kala azar."

Current Status

Leishmaniasis vaccination using living cultured organisms has been attempted since the early years of the 20th century, and field trials with a frozen whole organism **vaccine have recentl, been** undertaken **in Israel (138). Experimental studies in Australia using inactivated whole Leishmania (frozen and thawed infected culture cells) to infect genetically resistant and susceptible strains of mice are under way to devise standardized vaccination protocols. It has been demonstrated that avirulent parasite strains can induce protection in mice against related virulent strains (353). The theoretical possibilities of immunization are being developed and evaluated.**

Research Needs

In view of the large number of strains and types of *Leishnxmia* **and scientists' limited ability to identify organisms, there is doubt about whether a leishmaniasis vaccine will ever be practical. Each isolate is different. However, there is encouraging evidence from animal experiments and practical evidence of the potential for inducing active immunity. Further research on the current leads is needed.**

Filariasis

Filariasis is the collective term for several distinct parasitic infections by insect-transmitted, tissue-dwelling nematodes (roundworms). The most **important worldwide are** *Wuchereria bancrofti* **and** *Brugia malayi,* **the agents of filarial elephantiasis; and *Onchocerca volvulus,* the agent of onchocerciasis (river blindness) in west Africa, also found in Central and South America.**

Current Research

Until recently, filarial organisms were difficult to maintain for laboratory study, with complicated life cycles requiring insect vectors at one point. The lack of suitable animal models for many filarial diseases also hindered research. Investigators have achieved successful in vitro cultivation of *B. malayi* **and a related species** *B. pahangi* from the larval stage to young adult stage. They have also achieved some success with leaf monkeys *(Presbytis melalophos* **and P.** *cristata)* **as experimental** hosts (16).

Evidence of humoral immunity has been demonstrated by passive transfer of serum in mice and cattle previously inoculated with crude parasite preparations or live microfilariae.

Antifilarial MAbs are being produced for diagnostic purposes, but they can also be used for identification and purification of protective antigen. Attempts to identify specific antigens of possible significance to protection against *Wuchereria, Brugia,* **and** *Onchocerca* **are under way. At least one U.S. Government laboratory, one in**dustry laboratory, and one university laboratory are conducting or planning recombinant DNA work for expression of such filarial antigens (16).

Leprosy (Hansen% Disease)

Leprosy is the only bacterial disease among the six tropical diseases targeted by TDR. It is mainly a disease of skin and peripheral nerves, but is characterized by a wide array of clinical presentations.

The severe form of leprosy, lepromatous **leprosy, occurs in patients with defective cell-mediated immunity. These patients develop massive, contagious infections with extensive nerve damage. The nerve damage in severe cases seems to result from an overactive cell-mediated immune response. Most patients, however, develop a high**

level of cell-mediated immunity which kills and clears bacilli from the tissues. These patients, with tuberculoid leprosy, have a good prognosis.

The role of humoral immunity in leprosy still *is* not clear, but may be detrimental to the host. In the process of isolating *Mycobacteriwn leprae* bacilli from armadillo tissues, investigators have obtained significant quantities of a phenolic gly - colipid that is the only unique antigen present in *M. leprae* and not other mycobacteria. In vitro studies now indicate that immune reactions to this antigen may actually increase disease, without producing a protective effect (222). Characterization of the immune defect in lepromatous leprosy patients is being investigated using techniques of biotechnology and also by analysis of the patients' genetic makeup. Understanding the immune response to infection with *M. leprae* will help in developing immunization strategies.

Current Research

The tuberculosis vaccine BCG (an attenuated strain of a bovine mycobacterium) has been tested for an immunoprophylactic effect in large-scale prospective trials in Uganda, Burma, Papua New Guinea, and India. Long-term followup in these trials indicated a variable and not very effective protection against leprosy.

An interesting development in leprosy vaccination is the work of Convit and collaborators during the past decade in Venezuela (72). These researchers conducted clinical trials in a variety of individuals, including some with early disease, using a vaccine made of heat-killed *M. leprae (grown* in armadillos) combined with BCG vaccine. They found clinical, histopathologic, and immunologic changes in many patients with disease of moderate severity. The investigators' claim of conversion to effective cell-mediated immunity in lepromatous leprosy patients and more rapid clearance of bacilli in patients treated needs confirmation. Vaccine field trials using killed *M. leprae* only, BCG vaccine only, and *M. leprae* with BCG vaccine are planned. Such trials will require considerable amounts of M. *leprae.* **Since leprosy is a disease of low incidence and long incubation period, the evaluation of efficacy of a vaccine will require 10 years or more of observation in large populations.**

Carefully controlled human trials of leprosy vaccine from TDR are now under way in Norway in individuals never exposed to leprosy. It remains to be seen whether this vaccination will simply mimic natural exposure to leprosy (i.e., stimulating cell-mediated immunity in most people, without helping the smaller proportion of people who cannot mount such a response), or if it will support the earlier results of Convit and colleagues (72) of having some effect even in the lepromatous leprosy patient.

Extensive and careful planning of the production, purification, and testing of the leprosy vaccine, plus selection and epidemiological characterization of test populations has been under way in the TDR project for several years (349,350,353).

At least two groups are planning recombinant DNA work to try to make a leprosy vaccine, in order to eliminate the need for recovering *M. leprae* from armadillos (72). Some workers feel that subunit vaccines are not likely to confer significant protection, since they induce a humoral response but not cell-mediated immunity. One group of workers has isolated and characterized DNA from *M. leprae* and investigated its relationship to other mycobacteria (170). Further work may identify important DNA segments that could be cloned and used to produce quantities of a specific antigen.

Research Needs

Obtaining antigens from *M. leprae* grown in armadillos is a slow process and not suited to producing large quantities. In vitro culture methods are needed to facilitate leprosy research.

A concentrated effort is needed to use recombinant DNA methods and T-cell clones to sort out *M.* Ieprae-specific epitopes pertinent to protection. Better knowledge of cell-mediated immunity and the cellular aspects of host reactions is needed.

Further evaluation of BCG vaccine and *M. leprae* antigens through immunization trials is continuing. The fact that the tuberculosis vaccine (BCG vaccine) may prove to be useful against leprosy points out the importance of further study of the relationship of *M. leprae* to other mycobacteria, especially *M. tuberculosis.*

Tuberculosis

Tuberculosis remains a major threat to health in many parts of the world, causing several million deaths annually. Even in the United States, some 30,000 new cases of pulmonary tuberculosis were reported in 1980 (70). There is a tuberculosis vaccine, BCG vaccine, but its efficacy is uncertain and controversial. (For a review of immunology and microbiology of tuberculosis, see Collins (70) and Wayne (398).)

Current Status

BCG vaccine is an attenuated strain of a species of bovine mycobacterium. Since the early 1950s, BCG vaccination has been used extensively in tuberculosis control programs around the world. Even in the 1950s, however, it was known that BCG vaccine did not offer complete protection against tuberculosis. Results of controlled field trials were contradictory, with protection varying from O to 80 percent.

All the BCG vaccines used throughout the world today are derived from the original strain developed at the Pasteur Institute more than 50 years ago. There is no reliable method for standardization of BCG vaccines. Most people agree that the probable variation of different vaccine preparations, compounded by differences in immunologic response of populations, is the main reason for the enormous discrepancies in results between otherwise well-conducted BCG trials.

Because of the lack of definitive evidence of BCG'S efficacy, a controlled double-blind field trial in southern India was started in 1968 (414). Two highly ranked vaccines were used. After 7% years of careful followup, there was no evidence of a protective effect in BCG-vaccinated groups. The Indian field trial was meticulously reviewed (415,417) and found methodologically sound. Quite possibly, the explanation for the results in this trial is that the population had already developed some resistance to tuberculosis through exposure to a widespread south Indian nonhuman mycobacterium; and BCG vaccine could not add to that resistance. The results from this trial may not be applicable to other parts of the world, depending on local conditions. BCG vaccine is still considered useful in tuberculosis control programs.

Immunologic research on tuberculosis has been given relatively little attention over the years, and the field trial results in southern India clearly revealed a large gap in knowledge about the immunology of tuberculosis (417). Application of recombinant DNA methods to *M. tuberculosis* is in its infancy, but several investigators are planning projects. MAb work is under way in a handful of laboratories, including some at NIH and several academic institutions. MAbs are being reacted with *M. tuberculosis* to obtain purified antigen probes. Attempts are being made to isolate specific antigens for diagnostic skin tests (see ch. 8) in an attempt to make such tests less cross-reactive to infections with other types of mycobacteria, but such antigens may also have value for immunization.

Research Needs

A great deal of fundamental knowledge about tuberculosis immunology is needed to resolve the pending questions about immunization in general, the efficacy of BCG vaccine, and the susceptibility of various related mycobacteria to vaccination and their importance in the disease process.

Better understanding of tuberculosis immunology and vaccination and clarification of the effectiveness of BCG vaccine are still needed.

Diarrheal and Enteric Diseases

Diarrheal diseases are caused by a variety of viruses, bacteria, protozoa, and worms. Development of water and sanitation facilities where they do not now exist is a long-term solution to control these diseases. Nevertheless, some of the agents of diarrheal disease may well be susceptible to immunologic attack, thereby permitting prevention of disease with vaccines.

A few injectable vaccines inducing serum antibodies have been available for years (for cholera, typhoid, paratyphoid, *Shigella),* but these have limited effectiveness. Oral vaccines, which in most cases appear to induce a more appropriate immune reaction in the gut, are currently the focus of much research.

Although some research will have cross-over potential for several agents, it is generally expected

that each pathogen will need a tailored approach. Immunologic research has made it clear that the vaious etiologic agents evoke and are fought by a variety of immune responses. A further challenge of diarrheal disease research is the continuing discovery of new important etiologic agents.

Viral Infections

Rotaviruses cause about one-third of all diarrheal disease in the world and up to 50 percent of the hospitalized cases of diarrheal illness in children under 2 years (21,167,325,331). Animal rotaviruses are also important because of their economic significance in farm animals, and some veterinary vaccines have been developed and are in use.

Characterization of the rotavirus genome is being actively pursued with the aid of recombinant DNA techniques. Rotavirus RNA has been synthesized in vitro, reverse transcribed, and inserted into *Escherichia coli;* the genes from which most of the clones derive have been identified (117); bacterial clones containing copies of each rotaviral gene have been identified; some of the genes have been analyzed to determine the amino acid sequence of the proteins for which they code. This work is a promising means of identifying critical genes coding for the pathogenic characteristics or antigenic determinants of the organism. If successful, production of a strain of rotavirus with deletions of the pathogenic genes (similar to the cholera research described below) or transfer of the DNA segments that specify the antigenic determinant(s) and production of the antigen may be possible, forming the basis of a vaccine.

A major obstacle to vaccine development has been the lack, until recently, of a cell culture system for human rotaviruses (192,429). It may now be possible to develop attenuated vaccines through the various conventional methods (cell culture passage, cold adaptation, chemical mutagenesis, and reassortment) (371,385). In fact, volunteer studies in adults are being conducted to provide a test system for such candidate vaccines (181,182).

Animal rotaviruses have been successfully grown in culture, and attenuated strains are available as veterinary vaccines. Studies in calves, piglets, and lambs have demonstrated the importance of intestinal rotaviral antibody in preventing or attenuating illness. Because human and calf rotavirus strains share a common group antigen (179,711), calves inoculated in utero with bovine rotavirus were protected against challenge with human rotavirus. Furthermore, piglets infected with bovine rotavirus and later challenged with human rotavirus showed cross-protection (204,205).

On the basis of this evidence, an oral, live, attenuated bovine rotavirus vaccine was recently tested and found protective in a test population of human adults and children (385). The vaccine has now been tested and found to be safe and effective in protecting infants from natural rotavirus infection (386). This is a very promising development that is being tested further.

Bacterial Infections

Most of the vaccine research on the bacterial enteric pathogens is being done on enterotoxigenic *E. coli, Salmonella typhi* (the cause of typhoid fever), *Vibrio cholerae* (the cause of cholera), and Shigella (the cause of bacillary dysentery). Most research groups are studying several of these agents. Fewer laboratories are studying *Campylobacter, Yersinia,* other species of *Salmonella* and *V.brie,* and other pathogenic intestinal bacteria. Approaches to vaccine development are generally similar to those described below for *E. coli.* Progress against several of these agents is described below.

Coliform Infection.—Various strains of *E. coli* are now recognized as major causes of diarrheal disease in older children and adults living in developing countries. They also appear to be *a* chief cause of "traveler's diarrhea." The disease-causing characteristics of *E. coli* are under genetic control, and investigators in several laboratories are actively identifying and cloning the genes that code for attachment and colonization, virulence, toxin production, and antibiotic resistance. The goal of this work is the development of strains that stimulate specific immunity but are not themselves pathogenic. Successful development of vaccines against enterotoxigenic *E. coli* in animals has stimulated work towards a vaccine for humans (427).

Typhoid Fever.—Injectable, killed S. typhi vaccines against typhoid fever have proved to be protective for adults and older children living in endemic areas. These vaccines are unsatisfactory, however, because they frequently induce adverse reactions, they are not entirely protective, and they do not stimulate local intestinal immunity (419).

While oral, killed typhoid vaccines have provided only minimal protection in volunteer and field trials, several live attenuated S. typhi strains are under study and good progress has been made. A strain developed by the Swiss Serum and Vaccine Institute may well become available for widescale use very soon. This strain has been tested for stability, safety, and efficacy in volunteers (127), and in field trials in Egypt (391), all with positive results. The results of a trial in Chile, in collaboration with Walter Reed Army Institute of Research, unfortunately do not match the Egyptian results (24). The WHO Program for the Control of Diarrheal Diseases is planning for the use of this vaccine by collecting baseline information on the incidence of typhoid fever in different countries and reviewing other relevant data (423,427).

Cholera.—Cholera is relatively rare compared to the other diarrheal diseases, especially those caused by rotavirus and E. coli. However, the epidemic and pandemic potential of cholera is great enough to make cholera an important tropical disease. The severe diarrhea of cholera is caused by a toxin produced by *V. cholerae,* acting on the gut wall.

Although injectable, killed whole-cell and toxoid cholera vaccines are currently in use, these vaccines do not stimulate effective, long-lasting protection against cholera (47,86,200,201,260).

Scientists using traditional nonrecombinant mutagenesis have produced a number of attenu**ated mutant strains of *V. cholerae*** that do not produce the cholera toxin (nontoxigenic strains). These attenuated, nontoxigenic strains have been used experimentally as oral vaccines with some encouraging results, but genetic instability or poor colonizing ability in the gut make them unsuitable.

Researchers have very recently succeeded in using recombinant DNA techniques to produce a live, attentuated *V. cholerae* strain by deleting gene coding for the cholera toxin (178,223). A recombinant strain such as this could provide a vaccine that colonizes the gut and stimulates immunity without producing the toxin and without the capability of mutating back to the pathogenic type. Very early clinical studies are under way to assess the safety and efficacy of this new vaccine. Unfortunately, the *V. cholerae* with the genes for cholera toxin deleted cause diarrhea in volunteers, presumably from a different toxin (230).

Shigellosis.—Shigella *dysenteriae* is associated with serious disease and fatality (bacillary dysentery), but S. sonnei and S. *flexneri,* which cause less severe disease, account for a major portion of all isolates in diarrhea patients. The available injectable, killed vaccines are not effective, and oral vaccines have not been developed until recently. *Shigela* will colonize primates only, making work with animal models difficult and expensive. One group of researchers has taken an attenuated, live typhoid vaccine bacterium developed recently in Switzerland and has spliced into it the genes coding for production of an important S. *sonnei* antigen, thereby inducing immunity to both typhoid fever and S. *sonnei* dysentery to vaccinees (126,123). Testing in humans is getting under way. The same group has isolated genes for attachment factors in *Shigella* and inserted these through plasmid vectors into noninvasive E. *coli. These* novel recombination as well as a number of attenuated strains are under investigation as potential vaccines, but solid success is still elusive.

Acute Respiratory Infections (ARIs)

ARIs area heterogeneous mixture of diseases, caused by a variety of viruses, bacteria, and other agents, some of which also cause infections out-

side the respiratory system. As detailed below, some vaccines for ARIs are available, and progress is being made in developing new vaccines.

Viral Infections

Many viruses cause acute respiratory illness in humans. The following groups have been identified as most important (see Anderson, et al. (5) for a recent extensive review):

Influenza. -Outbreaks of influenza may occur annually, major epidemics every 2 to 3 years, and pandemics at 10- to 15-year (or more) intervals. The last pandemic (as of July 1985) was in 1968. In pandemics, mortality rates are devastating, particularly in the elderly. Three main types of influenza viruses, with numerous subtypes and strains, cause infection in humans. Infection sometimes confers long-term strain-specific immunity or partial immunity. Because of antigenic drift and shift, influenza vaccines must be reformulated every year against the predominant virus. The annual production of influenza vaccine targeted for the strain predicted to be prevalent is now well implemented in the United States. However, the need for annual renewal makes mass influenza vaccination in developing countries impractical at this time.

Knowledge of the chemical and antigenic structure of influenza virus has increased greatly and, combined with the application of current molecular biology, is leading to better influenza vaccines. More rational approaches to vaccine development have emerged, e.g., the discovery of chemicals able to release surface glycoproteins from the viral particle without affecting their antigenicity, thus producing an efficacious and safer (fewer side effects) killed vaccine; the use of “high yield” influenza A viruses are being used to produce seed strains for better vaccine production (264).

Inactivated influenza vaccines are in use in developed countries. Killed vaccines administered by injection are made from virus particles disrupted by chemical treatment. This treatment lowers the antigenicity but also reduces the adverse side effects. Purified vaccines that contain only the immunogenic antigen, free of nonimmunogenic proteins, and are well tolerated by children can be produced (198,347). The vaccines are usually reserved for selected high-risk populations, such as the elderly, chronic disease patients, or economically important public service groups. Although these vaccines do not provide complete protection (225) and their effectiveness is dependent on annual renewal according to the prevalent strain (163), the Centers for Disease Control has conducted field studies that show inactivated influenza vaccine to be extremely useful in preventing or attenuating influenza infection (54, 374).

Live, attenuated influenza vaccines for intranasal administration are being developed. The attenuated vaccines can be rapidly and reliably produced by the transfer of genes from a cold-adapted attenuated donor virus to any new wild-type influenza isolate. Live vaccines, produced by reassortant RNA, eliminate the pathogenic genes but retain the immunogenic surface antigens. Recent research has indicated that these vaccines may be superior to the inactivated vaccines (60). In a study in adult volunteers, the live, attenuated vaccine completely protected against illness, while the licensed inactivated vaccine did not. Furthermore the nasal administration (by nose drop) is more convenient for patient and medical personnel. Even with the live, attenuated vaccine, however, there is a need for annual administration. Longer term protection will be possible only if there are common antigens among strains.

Much effort by 8 to 10 laboratories in the United States is focused on the molecular biology of influenza. This reflects, to a large extent, the importance of influenza as a public health problem in the United States. With immunity under active study (76) and the practical value of immunization under review (271,304), hundreds of publications in recent years have resulted. A large number of genes have been identified and sequenced. Synthetic peptides have been found to be antigenic and are undergoing evaluation, but preliminary studies have shown that such vaccines have greatly diminished capacity to induce neutralizing antibodies. In an effort to understand virulence factors, investigators have applied innovative methods, such as “tryptic peptide fingerprinting,” “RNA-RNA hybridization,” and “oligonucleotide mapping” (16).

One interesting development is the cloning of influenza genes in vaccinia virus (the smallpox vaccine) and the subsequent expression of both vaccinia and influenza antigen resulting in simultaneous immunization of hamsters against both agents (319). Because the vaccinia virus is well characterized, and several extra genes can be spliced into its genome, there is speculation that this experimental work could be developed into a method for delivering several vaccines in one organism. Many safety questions need to be answered before the vaccinia vector approach can be evaluated fully. The rate of adverse reactions from smallpox vaccine is higher than those for vaccines in current use, and may not be acceptable when smallpox is not the target.

Respiratory Syncytial Virus (RSV).–The several known strains of RSV are the most important causes of lower respiratory disease (pneumonia and bronchiolitis) in children under 2 years. The disease tends to occur in sharp outbreaks. Previous immunization efforts have not been successful, but new techniques show promise. Cloning of surface proteins or production of genetically engineered attenuated vaccines may meet with success.

Early work using inactivated RSV showed that vaccinated children developed antibodies, but were not protected against infection (180,186). Various immunologic phenomena have been hypothesized and studied without clear resolution.

Vaccination with attenuated RSV administered through the respiratory tract has not been successful. The degree of attenuation is variable, and the vaccines are not very stable (49,286). Injecting wild-type RSV grown in cell culture induced antibodies in young children without causing symptoms of disease (0o). However, there is no evidence that RSV given by injection will reproduce, nor that the vaccine will not interfere with passively transferred maternal antibodies in infants. The lack of vaccine stability and the uncertainty of a viral replication after injection are important, because a virus that fails to reproduce may act as an inactivated antigen that can potentate disease due to natural infection, and because the greatest need for effective RSV immunization is in the first months of life when maternal antibodies are still circulating in the infant (passive immunity). Another important finding that may hamper vaccine development is the report of antigenic variation in a new strain of RSV, which means that any single vaccine may be ineffective against some RSV strains (154).

Parainfluenza. —There are four main types (serotypes) and two subtypes of parainfluenza viruses. These viruses are the second most common agents of ARI (after RSV), producing croup, bronchitis, pneumonia, and bronchiolitis in infants and children.

Vaccine research thus far has shown that serum antibodies circulating in the blood against type 1 virus do not protect against infection, but antibodies in nasal secretions directly correlate with resistance to reinfection (317). Vaccines of formalin-inactivated virus administered by injection induce high titers of neutralizing antibody without preventing infection, though severe illness is prevented. Results with experimental live, attenuated vaccines administered intranasally have been encouraging. Whether killed or attenuated virus is used, the usefulness of a parainfluenza vaccine would have to be measured by the vaccine's protective effect in children, especially infants, who often become infected during the first months of life and suffer the most severe symptoms. Establishing the safety of a vaccine will be difficult (198,264).

Adenoviruses. —At least 41 different types of adenoviruses have been identified as causes of ARI (especially a problem in military recruits in the United States), as well as of epidemic keratoconjunctivitis (an eye infection) and a venereal disease.

Vaccines to prevent adenovirus infection have been developed for use in military recruits. A live vaccine cultured in human cell culture has replaced the earlier killed vaccine, which was highly protective but was inconsistent in different batches. The live vaccine, containing two different serotypes responsible for adenovirus infection in military recruits, is given orally (264).

Unfortunately, the experience with the recruits is not directly transferable to the general population, for two fundamental reasons. First, the

strains of adenovirus that cause most disease in recruits are different from those prevalent in the population at large. Second, while oral administration works for recruits, it is unsuitable for children, because recruits, in general, do not pass the vaccine virus on to each other, but children do, and a good deal of disease can be caused in that way.

Rhinoviruses.—Rhinoviruses, with more than 100 serotypes, are the most common human ARI agents and the single most important cause of the common cold.

Inactivated rhinovirus vaccines have shown some protection when administered intranasally, but not by injection. Live, attenuated vaccines may provide more protection, but the large number of serotypes, the fact that rhinovirus strains may not completely lose their infectivity (51), the indications of antigenic variation (120), and the possible development of genetic reassortant variants as a result of dual infection all hamper the future development of rhinovirus vaccines (264). In spite of the problems, some commercial firms are working on the rhinoviruses.

Coronaviruses, Coxsackieviruses, Echoviruses.—These viruses are important causes of common cold-like illness in children and adults. Coronavirus disease is self-limiting (about 7 days) and will not be a priority for vaccination. Coxsackieviruses induce a resistance after infection that is long lasting. With many different types prevalent in different areas and the predominant type in any area varying every few years as immunity in the population rises, there will be obstacles to vaccination. Echoviruses share epidemiologic features of coxsackieviruses. Immunization does not seem to be a practical method of preventing infection because of the large number of serotypes (129,264). There currently is little, if any, vaccine-related research on this group of viruses (16).

Measles.—Measles is an ARI for which an excellent vaccine exists. The vaccine is widely used in the United States, as part of a measles eradication program, and in other parts of the world, in particular, as part of WHO's Expanded Program on Immunization (see ch. 5). Measles vaccination has been controversial in developing countries because of the variable seroconversion rate (percentage of vaccinated individuals who later have measurable antibodies) and protection rates (percentage of vaccinated individuals who do not get the disease). A problem that has impeded success of vaccination programs is that infants must be vaccinated in the narrow interval between the waning of maternally derived antibody and the time of exposure to natural measles.

Recent clinical trials of live, aerosolized measles virus vaccines (298,299,300) have demonstrated that the intranasal route of administration may be particularly useful in developing countries. In contrast to vaccines injected through the skin, the aerosolized vaccine can induce seroconversion in the presence of maternal antibodies, in infants as young as 4 months. The aerosolized vaccine raises the respiratory tract antibody level, offering better protection at the portal of entry rather than in the serum. It also simplifies the logistics of vaccine delivery. A recent study indicates that not all measles vaccines stimulate an equally high level of protection in humans, even though they meet potency standards at manufacture (301). Better definition of potency standards is required. Further clinical trials are needed to assess the true efficacy of the aerosolized measles vaccine (4). In addition, the practicality of delivering aerosol vaccines in developing countries requires investigation. Getting the dosage right with aerosols, for instance, is much more difficult than it is with other methods of vaccination.

Bacterial and Mycoplasmal Infections

Streptococcus pneumoniae.—S. *pneumoniae* causes pneumococcal pneumonia, a disease that can be fatal when complicated with bacteremia (bacteria in the bloodstream). S. ***pneumonia*** accounts for approximately 80 percent of all bacterial pneumonias; it is an important cause of hospital admissions in developing countries and an important cause of middle ear infections and bacterial meningitis (12,248,292).

Pneumococcal vaccine is available, and its protective effect is well characterized (356). Immunity is specific to each serotype (subgroup of a species characterized by common antigens), so vaccines must include the most common serotypes found in the area of use, The polyvalent formu-

lation used for the vaccine contains capsular polysaccharides from a number of pneumococcal types. The vaccine is ineffective in children, especially under 2 years. It is usually targeted to older people whose risk of infection and case-fatality rates are greatest. Some new techniques of conjugation show promise in improving effectiveness in young children, but a major problem is the lack of information about which strains are prevalent in developing countries.

Streptococcus pyogenes.—The approximately 70 serotypes of group A streptococci (S. pyogenes) cause a variety of diseases, including streptococcal sore throat, scarlet fever, and rheumatic heart disease. Attempts to produce a streptococcal vaccine have encountered two serious problems: cross-reaction of streptococcal antigens with heart tissue, and the large number of serotypes.

A synthetic peptide approach offers the possibility of eliminating cross-reacting antigens while preserving those components that confer immunity. A synthetic streptococcal vaccine has been successfully tested in animals, and development of a vaccine against rheumatic fever is possible within the next 5 years.

Bordetella pertussis. —Pertussis (whooping cough) is endemic worldwide, and epidemics also occur. Children under 5 years of age have the highest incidence of morbidity, and about 70 percent of mortality is among children less than 1 year old (21). Immunization is available with a killed pertussis vaccine that is highly effective. This pertussis vaccine is usually given in a triple vaccine known as DPT (diphtheria, pertussis, tetanus). The use of DPT vaccine among children is promoted through WHO's Expanded Program on Immunization (see ch. 5). Intensive research to reduce or eliminate the adverse reactions seen with the current vaccine is under way.

Hernophilus idhrenzae. -Immunization with the polysaccharide antigen of H. influenzae results in antibody response. Antibodies can also be induced by E. ***coli,*** which has a polysaccharide that cross reacts with H. ***influenza.*** Attempts are being made to increase the immunogenicity of the antigen by coupling it to proteins (264). Prospects for licensing a new vaccine that is immunogenic in infants and children look very good.

Mycoplasma pneumoniae. —M. pneumonia is now recognized as a major cause of primary atypical pneumonia. Infection induces serum antibodies, but pneumonia can occur in individuals with positive antibody titers. While vaccination studies seem to demonstrate that serum antibodies correlate with protection, this and the role of respiratory tract antibodies still are not fully understood. Although antibodies appear in young children (2 tos years old), peak incidence of disease is in older children and young adults.

Field trials with inactivated ***M. pneumonia*** vaccines have shown encouraging results, and attenuated vaccines have also been tested. However, some vaccinees have developed disease and have not produced detectable antibodies, and lung lesions may have resulted from an immune reaction. Further cautious work is needed to resolve these problems (50,264).

Arboviral and Related Viral Infections

Arboviruses include literally hundreds of distinct viruses that are widely distributed throughout the world. Arboviral infections such as yellow fever, dengue fever, and various types of encephalitis occur worldwide in endemic and epidemic forms.

Current Status

Vaccines are available for only a handful of arboviral infections. A live, attenuated vaccine for yellow fever (the 17D strain) was developed decades ago and is well established and successful in preventing yellow fever. It has proved to be extremely safe and effective. The immunity stimulated persists for more than 10 years. Yellow fever vaccine is grown in embryonated chicken eggs following classic procedures of viral vaccine production (373).

Formalin-inactivated vaccines are used against Japanese B encephalitis and Russian spring-summer encephalitis with good results. Experimental attenuated (Venezuelan equine encepha-

litis and western equine encephalitis) vaccines under research and development have been used in horses.

Research Needs

The most critical need is for prevention of dengue fever, not only because of dengue fever's debilitating clinical syndrome, but also because of dengue hemorrhagic fever (DHF), a serious complication of dengue infection that often leads to a shock syndrome and death (with symptoms similar to yellow fever).

Several laboratories, both military and civilian, are studying the biology of the four main serotypes of dengue virus. MAbs for early serotype-specific identification have been developed and are now generally available. These may also help to identify protective antigens. Attenuated vaccines are being developed using classical vaccine production techniques. Each of the four known serotype strains of dengue virus requires a separate vaccine. A serious question of vaccine efficacy relates to the origin of DHF. It is now believed that the sequential infection with two different dengue serotypes leads to DHF. It also appears that type 2 is the critical precipitator. Therefore, the hope is that vaccination against type 2 can prevent DHF; however, careful evaluation will be needed to ensure that vaccination will not lead to increased incidence of DHF as a side effect (143,144).

Very few vaccines exist against the arboviral agents, but previous successes argue for a continued research effort. Because there are literally hundreds of arboviruses that cause ill health, routine vaccination is still a long way off. Molecular characterization and increased taxonomic studies are needed to lead the way to "generic vaccines" (polyvalent) for large groups of related viruses, rather than serotype-specific vaccines. To develop vaccination strategies, there is also a need for better understanding of the epidemiology of arboviruses.

SUMMARY

Effective vaccines exist for some viral and bacterial diseases important in developing countries, but there are currently no antiparasite vaccines. The vaccines available in developing countries are largely those that are also used in the developed countries, mainly against childhood illnesses. Efforts to develop vaccines against rotavirus infection, which causes a large percentage of diarrheal illness, and malaria, one of the world's biggest health problems, are among the first efforts whose products will benefit mainly the developing countries, although there is some work in the direction of vaccine development for virtually every disease.

The successful application of immunization technologies, sadly, does not rest on exciting developments in research and development. The vaccines that are already available are far from universally applied because of financial and political constraints, and there is no reason to believe that new vaccines will fare any better unless they are supported by international and bilateral health programs.