Prospects for Vaccines to Protect Against AIDS, Tuberculosis, and Malaria

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Vaccination is perhaps the most powerful of all medical interventions. During the past 25 years, vaccination has eliminated smallpox worldwide, polio from the western hemisphere, and *Haemophilus influenzae* as a cause of life-threatening disease in North America and Europe. Prevention of infection by vaccination has improved the health status of human populations throughout the world. Despite this record of accomplishment, a virus (human immunodeficiency virus [HIV]), a bacterium (*Mycobacterium tuberculosis*), and a parasite (*Plasmodium falciparum*) now kill more than 5 million people annually.1 HIV and the acquired immunodeficiency syndrome (AIDS) have only become an epidemic in the recent past. Emergence of multidrug-resistant *M. tuberculosis* and the immunosuppression caused by HIV have dramatically increased the incidence of tuberculosis (TB) and death caused by TB throughout the world. Development of drug resistance by parasites and insecticide resistance in the *Anopheles* species mosquito, the deterioration of political and health care infrastructures in many parts of the world, movement of nonimmune refugee populations into malarious areas, and the population explosion in sub-Saharan Africa have all contributed to the increased incidence of malaria.

AIDS, TB, and malaria are more severe problems in poor countries than in affluent ones, and these diseases have not received an investment in research dollars commensurate with their importance. There is a growing realization of the impact of these diseases and an increasing belief that these diseases can be controlled by vaccination.

In this article, we describe the global impact of AIDS, TB, and malaria; summarize the current understanding of how the human immune system can be mobilized to contain the pathogens responsible for these diseases; and describe the approaches for vaccines that promise to eliminate these diseases.

**AIDS**

**Burden of Disease**

According to the Joint United Nations Programme on HIV/AIDS,2 more than 18.8 million people worldwide have died of AIDS and 34.3 million are infected with HIV, with 5.4 million people newly infected with HIV in 1999 alone. More than 13.2 million children have been orphaned by AIDS, and the disease has had a profound impact on rates of infant, child, and maternal mortality, life expectancy, and economic growth. In 16 countries, more than one tenth of the adult population aged 15 to 49 years is infected with HIV. In Botswana, 35.8% of adults are infected with HIV, and in South Africa, 19.9% are infected.2

Anti-HIV drug therapy will have a limited effect on containing the AIDS epidemic. Although available drugs decrease virus replication in infected individuals, the virus persists, even in those in whom anti-HIV therapy has eliminated measurable plasma virus. Ongoing viral replication will allow the emergence of drug-resistant HIV variants. More important, the real costs of providing antiretroviral drugs to the millions of individuals in need of treatment is beyond the financial resources of populations in the developing world. HIV can only be controlled worldwide by development of an effective vaccine.

**AIDS Pathogenesis and Immune Containment of HIV Spread**

HIV infection occurs most commonly worldwide as a result of venereal spread. While rare individuals have a relative
resistance to HIV infection, all people are ultimately susceptible to HIV infection. Preventing infection and the pathologic consequences of infection can be accomplished only through vaccination.

Although HIV causes immune dysfunction by several mechanisms, the central immune abnormality is a loss of CD4 T lymphocytes. Antibodies that recognize the envelope glycoproteins of HIV can neutralize the virus in vitro and block infection in a nonhuman primate. However, because of the extreme sequence variability of the envelope glycoproteins, such neutralizing antibodies are usually isolate-specific. The central role that CD8 cytotoxic T lymphocytes (CTLs) and CD4 T lymphocytes play in containing human HIV infections and monkey simian immunodeficiency virus (SIV) infections has been the subject of intense interest. During primary infection, HIV and SIV replication is contained by CTL responses. Potent CTL responses are associated with low virus loads and quiescence of clinical disease. CD8 lymphocyte–depleted monkeys are unable to contain SIV replication during primary or chronic phases of infection, and after virus challenge, SIV replication is contained in monkeys with vaccine-elicited CTL. Preservation of HIV-specific CD4 T-lymphocyte function correlates with containment of HIV replication in infected individuals. Taken together, these observations suggest that an effective HIV vaccine should elicit potent virus-specific CTL and CD4 T lymphocytes.

HIV Vaccine Strategies Being Explored

Several vaccine technologies elicit high-frequency CD4 T-cell and CD8 CTL responses. Live recombinant organisms are being explored as potential vaccines. In this approach, a gene encoding a protein of a pathogen can be inserted into an organism that infects humans but does not cause disease. This recombinant organism will then express the product of the inserted gene, and immunity to that gene product will be elicited as part of the immune response to the organism. Genes of HIV, M tuberculosis, and malaria have been inserted into such diverse vectors as pox viruses, bacille Calmette-Guérin (BCG), adenoviruses, and enteric bacteria. These approaches offer the possibility of eliciting the same long-lasting, potent immunity that is induced by infection with a live organism but without delivering a potentially pathogenic organism to the vaccinee.

Another approach for inducing T-cell responses is the direct injection of plasmid DNA expressing a gene encoding a protein antigen. After intramuscular or intradermal injection, DNA plasmids are taken up by cells and the encoded protein antigens are expressed. The proteins are processed by immune cells and generate strong, persistent cellular immune responses.

Other strategies under investigation combine 2 different vaccine technologies. The rationale for such “prime-boost” strategies is that the combination of 2 different immunization modalities that induce a cellular immune response by different mechanisms can be combined and synergize elicitation of T-cell immunity. “Prime-boost” approaches can also include 1 vaccine modality that elicits CTL and another that elicits neutralizing antibody responses. These approaches to vaccination are likely to provide the means of inducing meaningful immunity to these pathogens.

All of these strategies are under investigation as potential vaccines to prevent HIV infection. Because all potential vaccines cannot be evaluated for immunogenicity and efficacy in human populations, potential vaccines are evaluated in nonhuman primates, and the data from these studies are used to select the more promising approaches for early phase human testing.

Anticipated Vaccine Trials in Humans

Protection against challenge with highly pathogenic AIDS virus isolates has not yet been achieved in nonhuman primate studies. Nevertheless, data from nonhuman primate investigations suggest that eliciting T-cell immunity by vaccine prior to virus infection can alter the pathogenic consequences of infection. For example, previously vaccinated monkeys have no measurable virus in the plasma and no loss of CD4 T lymphocytes following infection. This raises the possibility that prior vaccination can reduce viral replication in humans subsequently infected with HIV. Such individuals are predicted to manifest decreased disease burden and decreased HIV transmission rates.

Plasmid DNA and recombinant pox strategies are being assessed in human clinical trials, and additional HIV vaccine strategies will be assessed in humans in the near term. However, until a vaccine can elicit antibodies that neutralize a diversity of HIV primary patient isolates, true protection against an infection might not be achieved. Improved virus-specific T-cell responses should contain viral replication and reduce disease manifestations among those who become infected.

Research Needs

Creation of an HIV vaccine is feasible, but a worldwide commitment is needed to develop such a vaccine. The industrialized nations of the world must commit the resources to develop this vaccine, and developing nations must create and provide the infrastructure to facilitate the testing of vaccine immunogens.

Tuberculosis

Burden of Disease

Each year, TB is responsible for 8 million new cases, 2 million deaths worldwide, and contributes to the deaths of an additional 900,000 people with AIDS. Thus, TB and AIDS are the largest causes of mortality from infectious diseases. Tuberculosis affects about 16 million people worldwide, with a case-fatality rate of 50% in untreated disease. In some countries with the highest prevalence of HIV coinfection, the fatality rate is about 23%. In an autopsy study in Africa, TB was the cause of death in 32% of AIDS deaths and a contributory cause in an additional 15%
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to 25%.12 The peak age of incidence of TB is 15 to 25 years of age, but infection can persist, often for a lifetime, in a silent form, and can reactivate with HIV infection.13 Only 1 in 10 individuals infected with M tuberculosis, determined by tuberculin skin testing, develops disease within a lifetime, whereas the risk is about 8% per year in immunodeficient individuals. This suggests that most infected individuals are protected by an immune response and that enhancement of the natural immune responses could increase resistance to disease. Epidemiological models predict that even a 50% effective TB vaccine would have a major impact on the disease and would save perhaps 40 million lives over a decade.14,15

Current Treatment
Perhaps due to its unusual waxy coat, the tubercle bacillus is impervious to most antibiotics. Resistance develops quickly to single drugs, and a complex regimen known as directly observed treatment, short course (DOTS) requires administration of 3 to 4 drugs for the initial 2 months of treatment, followed by 2 drugs for 4 to 7 months.16,17 The complete DOTS regimen is accessible to only 15% of patients worldwide, and multidrug-resistant TB has emerged and is extremely difficult and expensive to control. Globalization and migration represent threats for transmission of drug-resistant TB to the United States. Drug-resistant TB is a major threat and emphasizes the need for new tools for prevention and treatment.

Current Vaccine
The BCG vaccine, an attenuated strain of Mycobacterium bovis discovered in 1908 and first used in humans in 1921, is the most widely used vaccine in the world, being administered currently to about 104 million children. The BCG vaccine clearly prevents death caused by disseminated TB and TB meningitis in children, but its effectiveness in adults varies.18 For example, in a large prospective trial in the United Kingdom, BCG resulted in 77% protection for teenagers,19 whereas a similar trial in India was not protective in any age group20; other trials have efficacies between those extremes. The reasons for this wide variability are not fully clear. Since children represent only 10% of TB cases, the impact of BCG on the epidemiology of TB in adults has not been great.

Mechanisms of Pathogenesis and Protection in TB
The lower lung is the target of primary infection by M tuberculosis, but the bacilli spread hematogenously to the apex of the lungs or to other organs where disease becomes manifest. In animals, BCG does not block infection, but limits hematogenous spread and disease. The immune mechanisms that provide protection against TB have not been identified. Most evidence indicates that cell-mediated immunity is essential for protection and that antibodies play little role. Mice with targeted disruptions of immunologically important functions, eg, deficient in CD4 T cells and in the ability to produce interferon-gamma, become highly susceptible to TB, indicating that lymphokines and macrophage activation are essential for protection. Cytotoxic T lymphocytes may also be required.

Candidate Vaccines
More than 100 vaccine candidates have been tested in animal models. With the genome sequence of M tuberculosis complete, and that of BCG and nonpathogenic mycobacteria in process, additional targets for vaccines are certain to emerge. Potential vaccine concepts include the following:21-23:

Subunit vaccines, consisting of mycobacterial protein, lipid, and carbohydrate antigens in various formulations, have the potential to be specific, defined, and safe. Their disadvantage is limited persistence in vivo and the nature and duration of the immune responses they generate.

DNA vaccines that encode several M tuberculosis antigens are protective in mice. These are easy to produce, relatively inexpensive, and induce long-lasting cell-mediated immune responses. Formulation of the DNA in adjuvants and alteration of the composition of DNA increase immunogenicity. Safety and duration of protection are not yet defined. One promising report indicates that a DNA vaccine encoding the 65-kd antigen was able to abrogate persistence or latency in a mouse model, which live BCG was unable to do.24

Nonmycobacterial microbial vectors such as engineered Salmonella and vaccinia organisms that express mycobacterial antigens are being tested. The former has the potential to induce mucosal immunity, and the latter can induce CTLs.

Live attenuated mycobacterial vaccines, including nonpathogenic mycobacterial species and genetically engineered BCG expressing immunodominant antigens of M tuberculosis, are under development. In a UK trial, Mycobacterium microti was as effective as BCG in protection, even though it produced positive skin test conversion in only a fraction of the vaccinees. In addition, genetically attenuated strains of M tuberculosis, including auxotrophic mutants and mutations in genes relevant to persistence and virulence are under development and some appear to be effective in mice. They have the advantage of containing a wide range of antigens, the adjuvanticity associated with mycobacteria and persistence, but safety in immunodeficient individuals has not been established.

Trial Designs
There are 4 general strategies to test safe and immunogenic vaccines for efficacy in humans:

Preinfection. Infants at high risk for early infection and disseminated disease or meningitis would be vaccinated close to birth, and the impact on prevention of infection, acute disease, or persistence would be assessed, perhaps over a 3- to 5-year period. It is not known whether protection against acute disease in young children would predict efficacy in teenagers and adults.

Postinfection. The target population is tuberculin-positive healthy adults, in areas with a high reactivation rate, eg, 3% per year. Diminution of reactivation would be assessed over 3 to 5 years.
It is unclear whether results of trials in individuals already immunized by natural infection will predict protection in unimmunized individuals.

Prospective Trial in Uninfected Individuals. From experience with BCG trials, this design would require large numbers of individuals who would have to be followed up for 15 to 20 years, but would provide the most unambiguous resolution of vaccine efficacy.

Total Population Trial. An epidemiologically characterized population would be immunized. Those already tuberculin positive would be analyzed for protection in 3 to 5 years, and the efficacy among uninfected individuals could be ascertained in the same trial over a longer period of time.

Research Needs
Since efficacy trials are long, complex, and expensive, surrogate markers that correlate with immunologic protection are needed. These markers, whether lymphokines, CTLs, or mycobactericidal activities, if measurable rapidly and quantitatively, would accelerate vaccine development and testing.

Although TB occurs in every country of the world, more than 80% of cases occur in developing countries. This limits the market for TB vaccines greatly and is a major disincentive to the pharmaceutical industry to develop them. To make the investment by industry in vaccine development feasible, there is a need for public-private collaboration, in which public sector investments move the research forward (“push”) and ensure markets or purchase of vaccines found to be effective (“pull”).

Finally, the collaboration of developing countries where the disease is most prevalent will be essential to evaluate the safety and efficacy of TB vaccines.

MALARIA
Scope of the Problem
The mortality rate of children with severe malaria in primary care hospitals in the developing nations has not been reduced in 25 years. In many parts of the world, malaria is more common today than 25 years ago. Malaria accounts for an estimated 300 million to 500 million new infections and 1 million to 3 million deaths annually and is thought to reduce the annual gross domestic product in sub-Saharan Africa by 1% to 4%. There is currently no malaria vaccine, and there is little prospect for deployment of an effective vaccine in the next 5 years against any of the malaria parasites that infect humans.

Advances and Scientific Foundation
Parasites that cause malaria are more complex than the viruses and bacteria for which vaccines are available, making vaccine development difficult. They have approximately 6000 genes, and a multistage life cycle with stage-specific expression of many proteins at each stage. This means that antibodies against a protein on sporozoites (the stage inoculated by mosquitoes) will generally not recognize the major protein on the surface of erythrocytic stage merozoites. A single individual can be infected with more than 5 different strains of P falciparum (allelic variation), and one of the proteins expressed on the surface of the infected erythrocytes, a protein that is important in disease pathogenesis, can evade antibody responses by expressing 50 to 100 antigenically different variants.

In contrast to vaccines for other diseases, several types of malaria vaccines can be envisioned. One for nonimmune travelers would prevent infection of erythrocytes, thereby preventing all clinical manifestations (type 1). A second type for young children in sub-Saharan Africa would limit replication at the erythrocytic stage without preventing infection, thereby preventing the 1 million to 3 million deaths caused by malaria annually (type 2). There is also interest in developing a malaria vaccine directed to the sexual phase of parasite reproduction that would not protect the individual, but would reduce transmission of infection within a community, thereby reducing disease burden.

Development of malaria vaccines is believed to be feasible because human models are available for the approaches. Immunization of volunteers by exposure to the bites of more than 1000 irradiated, infected Anopheles species mosquitoes provides more than 95% protection for up to 9 months against experimental challenge with multiple strains of P falciparum. The irradiated sporozoite vaccine would be an ideal type 1 vaccine, but is impractical. Children in areas of sub-Saharan Africa with intense malaria transmission who live until the age of 8 to 10 years do not develop severe malaria, but do become infected and develop febrile illness. These children have an immune response that does not prevent infection but limits the pathological and clinical effects of the infection. A vaccine that essentially turns infants and young children into 10-year-olds from an immunological standpoint would be a candidate type 2 vaccine.

The challenge has been to characterize immune responses that provide protection, to define which parasite antigens/epitopes are targets of these protective immune responses, and to develop vaccine delivery systems that induce the appropriate immune responses. Irradiated sporozoite-induced protection against malaria in mice is primarily mediated by CD8 T cells that recognize 8 to 10 amino acid peptides derived from the parasite on the surface of infected hepatocytes. This immunity is complemented by antibodies that prevent sporozoites from invading hepatocytes and by CD4 T-cell responses against the proteins/epitopes expressed at the liver stage. Other evidence suggests that naturally acquired immunity in residents of malaria endemic areas is primarily mediated by antibodies that recognize parasite proteins expressed on the surface of erythrocytic stage merozoites or on the surface of infected erythrocytes. T-cell responses at the erythrocytic stage, antibodies against sporozoites, and T-cell responses against infected hepatocytes are also thought to play a role.

Current Cutting-Edge Research Activities and Critical Events
Three major approaches are being pursued to attempt to create a multi-antigen, multistage malaria vaccine.
The first is designed to enhance antibody and CD4 T-cell responses against a few key proteins, on sporozoites, merozoites, and sexual stages using purified proteins or peptides administered in a strong adjuvant. The most progress has been made with the major surface protein of sporozoites, the \textit{P falciparum} circumsporozoite protein. A vaccine formulation known as RTS,S/SBA52 consistently protects 50% of volunteers against experimental malaria challenge for 2 to 3 weeks, but not for 6 months, \textsuperscript{30} and among Gambian semi-immune adults in The Gambia, provided approxi- mately 65% protection for 2 months and no protection at 6 months. This repro- ducible but unsustained protection is a step forward, but is not adequate and this vaccine alone most likely will not re- duce mortality in infants and young children in the developing world. A protein on the surface of erythrocytic stage merozoites (merozoite surface protein 1 [MSP1])\textsuperscript{31} is also a component of the experimental vaccine, SPf66,\textsuperscript{32} which de- spite early promise has not been effec- tive in field trials in The Gambia, Thailand, Tanzania, and Brazil; MSP1 is also part of a trivalent vaccine recently shown to be promising in Papua, New Guinea. Recombinant \textit{P falciparum} MSP1 will be tested in volunteers, both alone and in combination with other proteins like the \textit{P falciparum} circumsporozoite protein, and at least 5 other asexual and sexual (transmission blocking) erythrocytic stage–derived recombinant pro- teins will be tested in the near future.\textsuperscript{27}

The second approach is designed to induce antibody and CD8 and CD4 T-cell responses against proteins expressed by irradiated sporozoites in hepa- tocytes (n = 5) and proteins expressed on or near the surface of erythrocytic stage merozoites (n = 10). This approach did not seem feasible using pu- rified recombinant proteins, but DNA vaccines make it possible to devise and evaluate multistage, multiantigen vac- cines. Early studies indicate that CTLs\textsuperscript{33} and interferon gamma–producing CD8 T lymphocytes can be elicited in human volunteers. However, while DNA vaccines appear to prime the immune system, boosting the immune re- sponse with recombinant viruses and recombinant proteins appear to pro- vide more protective immunity than DNA immunization alone.\textsuperscript{34} Clinical tri- als are under way with multiple liver stage genes as DNA vaccines alone and in a prime-boost strategy.\textsuperscript{35,36}

The third approach uses data from the Malaria Genome Sequencing Project. Irradiated sporozoite and natu- ral immunity is induced by exposure to the entire parasite and could reflect im- mune responses against many pro- teins encoded by the estimated 6000 genes in the \textit{P falciparum} genome. The genomic sequence of \textit{P falciparum} will be completed by the end of 2002,\textsuperscript{37,38} and a variety of techniques are being used to identify new targets for vac-
vaccine development. Using the genetic sequence for development of successful vaccines will require new approaches to constructing DNA-based and polypeptide vaccines. Clinical trials of such vaccines could begin within 3 to 5 years.

**Critical Elements and the Future**

The human models (irradiated sporozoite and naturally acquired immunity) demonstrate the feasibility of a malaria vaccine, and developments in genomics, proteomics, molecular immunology, vaccinology, population genetics, and quantitative epidemiology have created great expectations for development of effective malaria vaccines. It will be a formidable task to determine which antigens/epitopes from which stages of the life cycle of the malaria parasite are required for sustainable protection, which immune responses predict protection, which vaccine delivery systems are optimal, who and when in life to immunize, and the true impact of a malaria vaccine. However, the next 10 to 25 years should see the development of effective malaria vaccines that will mitigate the effects of the disease worldwide and, combined with other interventions, will eradicate malaria in many areas.

**CONCLUSION**

AIDS, TB, and malaria are caused by different pathogens that differ in many ways. Nevertheless, each elicits a profound immune response and all 3 diseases could be successfully contained and perhaps eliminated through vaccination. Cell-mediated immunity can play a central role in controlling these infections. Several novel vaccine strategies elicit durable cell-mediated immune responses that appear to be able to contain these infections. These diseases are important public health problems in the developing world, and the affected nations must provide the health care infrastructure to deal with them. Advances in understanding of these diseases and immunization technology suggest that vaccine protection against HIV, M tuberculosis, and malaria parasites are achievable in the coming decades. Attaining these goals requires a concerted commitment of scientific and economic resources to these public health problems.

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**REFERENCES**