VACCINE UPDATES

Dengue

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Dengue is a mosquito-borne infection that in the 1950s affected only a few countries in Southeast Asia and Latin America [1]. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia, and the Western Pacific. The World Health Organization estimates that approximately two-fifths of the world 's population is at risk of dengue infection [2]. Dengue also has started to cause outbreaks in the United States (Hawaii in 2001, Texas in 2005, and Florida in 2010) after having been absent from the country for more than 50 years. The reemergence of dengue in many parts of the world is believed to have been caused by increased urbanization and international travel and by climate changes that have affected the habitat and geographical distribution of the *Aedes* mosquitoes that spread dengue virus.

Dengue infections are caused by four different virus serotypes (DENV–1, –2, –3, and –4). The majority of dengue infections are either asymptomatic or result in a mild, self-limiting influenza-like illness called dengue fever (DF). In some cases, dengue infection results in severe disease—dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS)—which causes significant morbidity and mortality, especially in children [2].

The risk factors for developing severe dengue disease are not yet understood, but it is believed that pathogenic immune responses play an important role. Epidemiological studies have shown that the majority of DHF/DSS cases occur in secondary infections with a different serotype or in infants born to DENV-seropositive mothers. There are two main theories to explain these observations. In the first theory, antibodies produced in response to the initial infection do not neutralize the second heterotypic infection, but instead form a complex with the virus and enhance the infection by facilitating entry into Fc receptor-bearing cells. This phenomenon is called antibody-dependent enhancement (ADE) [3]. ADE has recently been demonstrated in mouse models of dengue disease [4, 5]. In the second theory, severe dengue disease is caused by pathogenic cytokines that are produced by infected T cells in response to a secondary infection with a different viral serotype. In this theory, proposed to explain severe disease in older children and adults, the secondary infection with a different serotype induces a memory T-cell response that has low affinity for the second virus and results in altered T-cell functional responses and dysfunctional cytokine production that can cause disease. Studies in human infections and animal models have provided evidence for this theory [6]. It is likely that both antibodies and T cells play a role in disease development.

Vaccines for dengue are not currently available, though research to develop a vaccine has been ongoing since the 1930s. There are several factors that have impaired the development of a dengue vaccine. First, an ideal dengue vaccine should confer strong and long-lasting neutralizing immunity against all four dengue serotypes. Partially protective or short-lasting immunity induced by dengue vaccines has the potential to cause enhanced disease if vaccine recipients are subsequently exposed to infection [7]. This potential risk has made the evaluation of dengue vaccines in endemic countries difficult. Second, there are no good animal models that recapitulate human dengue disease, and therefore it has been difficult to measure the attenuation of live vaccines and vaccine efficacy before evaluating them in humans [8]. Third, it can be difficult to achieve balanced immune responses against all serotypes in tetravalent live-attenuated vaccines, as the individual virus components of the vaccines can interfere with each other [9]. Despite these difficulties, significant progress has been made in the last few years toward developing a vaccine, and the research community is now closer than ever before to having an approved dengue vaccine on the market.

Currently, the vaccine that is most advanced in development is the ChimeriVax dengue vaccine developed first by Acambis and more recently by Sanofi Pasteur. This vaccine is a mix of four recombinant, live-attenuated yellow fever 17D vaccine viruses, each one expressing the premembrane (prM) and envelope (E) genes of one of the four dengue serotypes. This vaccine has been tested in several Phase I and Phase II clinical trials in the United States, Asia, and Latin America, in both adults and children. After three doses given 6 months apart, the vaccine confers balanced immune responses against all four serotypes and seems to be well tolerated [9]. Phase

III trials of this vaccine started in Australia in 2010 and are currently ongoing.

Three other vaccines are currently in clinical development:

1. The Laboratory of Infectious Diseases (LID) at the National Institute of Allergy and Infectious Diseases (NIAID) is developing a similar tetravalent, recombinant, live-attenuated dengue vaccine, based on an attenuated DENV-4 rather than a yellow fever 17D "backbone." LID used several novel methods to discover mutations capable of attenuating dengue virus [10]:

    - Researchers followed a reverse genetics approach to remove a stretch of 30 nucleotides shared by all serotypes in the untranslated region (UTR) of the genome. This mutation (Δ30) was attenuating and genetically stable, thus making the tetravalent vaccine safer by preventing viruses from reverting to virulent form.

    - Researchers made use of a chemical mutagenesis screen that produced an extensive collection of mutated dengue virus strains, some of which presented useful characteristics, including attenuated replication. DNA sequencing of these virus strains identified the attenuating genetic changes that would be useful for engineering a live-attenuated dengue vaccine.

    - Researchers continued improving on the original delta 30 modification by removing additional nucleotides from the UTR and by swapping UTRs bearing delta 30 between different serotypes.

    Following identification of a suitably attenuated DENV-4, LID used this strain as the background to create chimeric viruses in which the structural genes were replaced with those derived from the other three serotypes. Using a combination of these techniques, LID was able to achieve optimal levels of attenuation and immunogenicity for all four serotypes. These attenuated viruses are presently being evaluated in human trials and already have shown evidence of being safe and immunogenic. Seven LID Phase I clinical trials in the United States have evaluated different monovalent formulations to find the best candidates for use in a tetravalent formulation. In 2010, LID initiated Phase I clinical trials of four different combinations of tetravalent vaccine to determine the best formulation to induce balanced immune responses against all four serotypes. Because vaccine strains also were selected for their ability to grow well in cultured cells, the cost of manufacture should be low, thus making the vaccine attractive to developing countries in dengue-endemic areas. This vaccine technology has been licensed to industry partners in Brazil, India, and Vietnam for further development.

2. A different tetravalent, recombinant, live-attenuated vaccine is currently being developed by InViragen [11]. The backbone for this vaccine is an attenuated DENV–2 strain (PDK–53) developed by the Centers for Disease Control and Prevention that was shown to be safe and immunogenic in Phase I clinical trials. The structural genes (prM and E) of this virus have been replaced with those of the other three strains. The tetravalent vaccine is a mixture of four viruses: PDK–53 and PDK–53 expressing the structural proteins of DENV–1, DENV–3, and DENV–4. This vaccine has been shown to be safe and immunogenic in animal models. In 2010, InViragen initiated clinical evaluation of this vaccine in two Phase I trials: one in the United States (through the NIAID Vaccine and Treatment Evaluation Units) and one in Colombia. Preliminary data suggest that this vaccine is well-tolerated and immunogenic in healthy adults.

3. Another vaccine, a recombinant subunit vaccine based on the truncated form of the dengue E glycoprotein (80E), originally was developed by Hawaii Biotech and is now being developed by Merck [12]. This vaccine is produced in *Drosophila* cells and has been shown to be safe and effective in preclinical studies. In 2009, a monovalent DENV–1 vaccine formulated with alum adjuvant was evaluated in a double-blind, placebo-controlled, dose-escalation safety study in healthy people. Preliminary results show that this vaccine is well-tolerated and immunogenic. The vaccine is now being reformulated with Merck's proprietary saponin-based adjuvants. Plans for further clinical development are being discussed.

    Several additional vaccine candidates using a wide variety of approaches are currently in preclinical development. These include inactivated whole virus particles, viral expression vectors such as Venezuelan equine encephalitis replicon vectors and adenoviruses, DNA-based vaccines, epitope-based vaccines, and immunogenic fragments of recombinant E glycoprotein with a variety of adjuvants.

REFERENCES

1. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol. 2002 Feb;10(2):100-3.
2. World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva: World Health Organization; 1997.
3. Halstead SB. Neutralization and antibody-dependent enhancement of dengue viruses. Adv Virus Res. 2003;60:421-67.
4. Balsitis SJ, Williams KL, Lachica R, Flores D, Kyle JL, Mehlhop E, et al. Lethal antibody enhancement of dengue disease in mice is prevented by Fc modification. PLoS Pathog. 2010 Feb 12;6(2):e1000790.
5. Zellweger RM, Prestwood TR, Shresta S. Enhanced infection of liver sinusoidal endothelial cells in a mouse model of antibody-induced severe dengue disease. Cell Host Microbe. 2010 Feb 18;7(2):128-39.
6. Mathew A, Rothman AL. Understanding the contribution of cellular immunity to dengue disease pathogenesis. Immunol Rev. 2008 Oct;225:300-13.
7. Murphy BR, Whitehead SS. Immune response to dengue virus and prospects for a vaccine. Annu Rev Immunol. 2011 Apr 23;29:587-619.
8. Cassetti MC, Durbin A, Harris E, Rico-Hesse R, Roehrig J, Rothman A, et al. Report of an NIAID workshop on dengue animal models. Vaccine. 2010 Jun 11;28(26):4229-34. Epub 2010 Apr 29.
9. Guy B, Barrere B, Malinowski C, Saville M, Teyssou R, Lang J. From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. Vaccine. 2011 Sep 23;29(42):7229-41. Epub 2011 Jul 13.
10. Blaney JE Jr, Durbin AP, Murphy BR, Whitehead SS. Targeted mutagenesis as a rational approach to dengue virus vaccine development. Curr Top Microbiol Immunol. 2010;338:145-58.
11. Osorio JE, Huang CY, Kinney RM, Stinchcomb DT. Development of DENVax: a chimeric dengue-2 PDK-53-based tetravalent vaccine for protection against dengue fever. Vaccine. 2011 Sep 23;29(42):7251-60. Epub 2011 Jul 21.
12. Coller BA, Clements DE, Bett AJ, Sagar SL, Ter Meulen JH. The development of recombinant subunit envelope-based vaccines to protect against dengue virus induced disease. Vaccine. 2011 Sep 23;29(42):7267-75. Epub 2011 Jul 21.

VACCINE AGAINST CHIKUNGUNYA VIRUS IN DEVELOPMENT

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The National Institute of Allergy and Infectious Diseases (NIAID) soon hopes to launch a Phase I clinical trial of a candidate vaccine for chikungunya virus, a mosquito-borne pathogen that has infected millions of people, primarily in Africa and Asia, and causes debilitating pain. Researchers at NIAID's Vaccine Research Center (VRC) developed the vaccine and are making pharmaceutical-quality supplies of it in the VRC production facility for their clinical research. Phase I trial objectives include examination of vaccine safety and tolerability and early assessment of the immune response.

The vaccine uses virus-like particles (VLPs) to elicit an immune response. VLPs essentially present the outer surface of chikungunya virus, but lack DNA and therefore pose no infection risk. VRC studies in mice and nonhuman primates have shown that immunization with the candidate vaccine produces antibodies that can protect against a live virus challenge, even one nearly 4 months after immunization.

There presently is no vaccine or treatment for chikungunya virus infection. Chikungunya was isolated in Tanzania during the early 1950s. The name is derived from a tribal dialect word that means "that which bends up," reflecting the contorted posture of chikungunya patients suffering severe joint pain as a result of the disease. The joint pain can be incapacitating and long-lasting.

VLP vaccines are relatively new: The Food and Drug Administration has approved one for hepatitis B virus and one for human papillomavirus. The VRC work marks the first time scientists have used VLPs in a vaccine to protect against chikungunya virus, which is in the genus *Alphavirus*. The VRC scientists plan to determine whether VLP vaccines also will work against other alphaviruses, such as Western and Eastern equine encephalitis viruses found in the United States and o'nyong-nyong virus found in Africa.

Severe Acute Respiratory Syndrome

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Background

In the spring of 2003, the world first learned of an outbreak of a newly recognized atypical pneumonia that was subsequently named severe acute respiratory syndrome (SARS). Believed to have originated in the Guangdong province of China in late 2002, SARS quickly spread to Hong Kong, Taiwan, Singapore, Canada, Vietnam, and, ultimately, to a total of 29 countries. Overall, the World Health Organization reported 8,096 probable cases of SARS and 774 fatalities in less than 1 year; 27 of those cases were in the United States [1].

The speed with which the global health community responded to SARS was unparalleled. Shortly after SARS first emerged, the disease's etiological agent was identified as a novel coronavirus called SARS–CoV, which was determined to be phylogenetically distinct from previously known human and animal coronaviruses [2]. Characterization of the virus indicated that it was a single-stranded, positive-sense RNA virus, with a large genome of 29.7 kilobases.

SARS–CoV was discovered to be primarily transmitted by close contact from person to person via large respiratory droplets. Initial signs of illness included flu-like symptoms, with fever, cough, body aches, and malaise after an incubation period ranging from 3 to 10 days. Most patients developed pneumonia, and more than 60 percent of chest X-rays showed infiltrates. Up to 20 percent of individuals had diarrhea.

Epidemiological investigations showed that SARS disproportionately affected healthcare workers and close contacts of SARS patients, such as family members. Higher mortality was observed in older patients, with more than 50 percent of fatalities occurring in people 65 years of age or older. Children were the least likely to develop the disease [3].

The SARS–CoV outbreak likely originated in a few exotic animals in Guangdong marketplaces. SARS–CoV-like viruses, with 99 percent identity to human strains, were isolated primarily from Himalayan palm civets as well as other marketplace animals. From two independent field studies, another animal species, the Chinese horseshoe bat, was subsequently found to harbor a SARS–CoV-like virus that was 93 percent identical to human SARS–CoV [4, 5]. Because SARS–CoV-like virus was not found in wild or farm-raised palm civets, it is thought that the horseshoe bat may serve as the natural reservoir of the virus, with the civet serving as the intermediate host. Both animals were sold in Chinese wet markets.

Months after the disease first emerged in mainland China, the clinical syndrome was characterized, the etiological agent was identified, diagnostic tests were developed, and the virus genome was completely sequenced. The speed of scientific understanding and information exchange, combined with critical public health measures such as patient isolation and infection control, eventually led to successful outbreak containment. In July 2003, the World Health Organization officially declared the outbreak over. Since then there have been four separate laboratory-acquired SARS infections—one each in Singapore and Taiwan, and two in China. In addition, two individuals in southern China contracted SARS in December 2003 related to restaurant exposures.

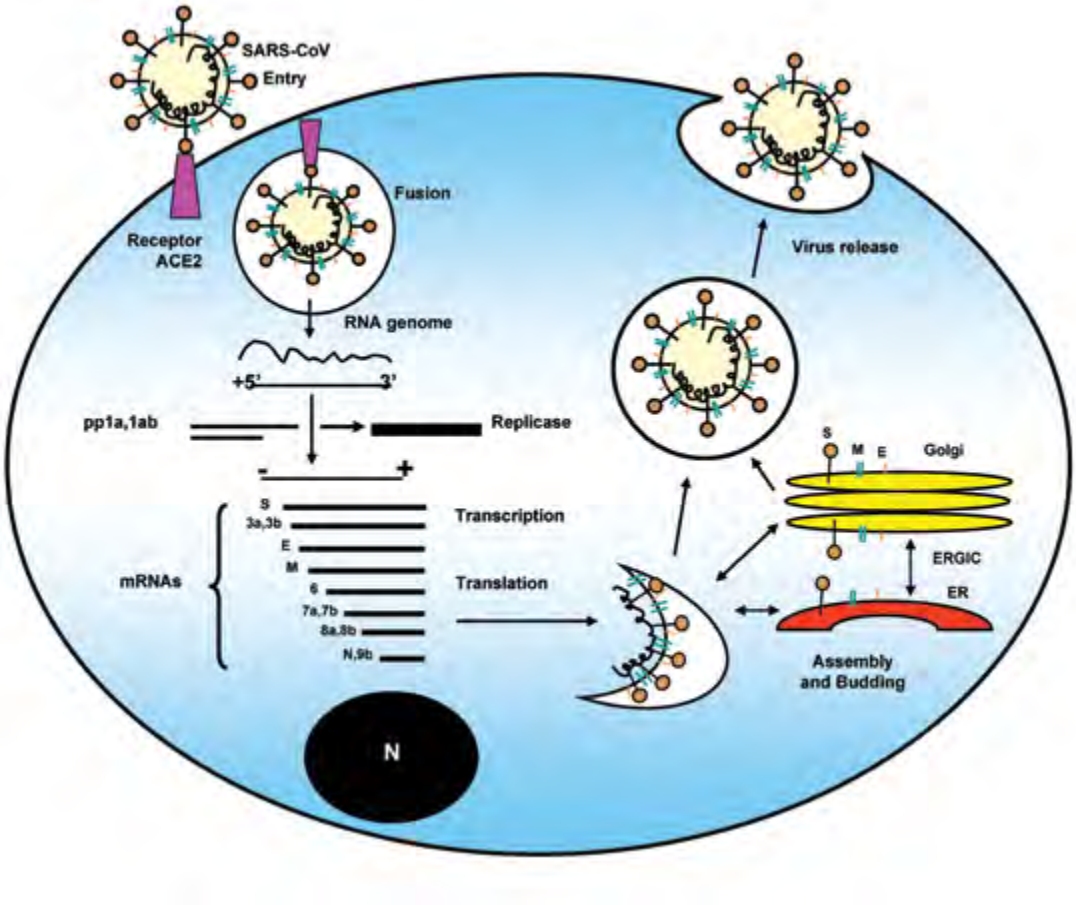
There have been no new SARS cases reported since April 29, 2004. Although the 2003 outbreak has not been repeated, the threat has not disappeared, because an animal reservoir of the precursor virus exists in nature and there is the possibility of an accidental or intentional release of the virus. The population in general, and SARS–CoV researchers specifically, remain at risk without any available prophylactic or therapeutic. Although the global health impact of the SARS 2003–2004 outbreak was tremendous, it paled in comparison to the global economic impact with respect to travel, tourism, and service industries.

SARS Research, Development, and Clinical Testing

National Institute of Allergy and Infectious Diseases (NIAID)-supported scientists have made significant advances in understanding SARS–CoV and its pathogenicity (Figure 1). For example, researchers have identified and characterized the lung receptor molecule, angiotensin converting enzyme-2 (ACE2), to which the S protein adheres [6]. Regions of interaction between the S protein and ACE2 have been mapped and characterized, and the domains of the S protein necessary for viral infection have been determined [7]. This is particularly important in designing improved candidate vaccines and therapeutics. Researchers have learned that the entry of SARS–CoV is blocked by inhibitors of the endosomal protease

FIGURE 1.

SARS–CoV life cycle



SARS–CoV binds to the target cell via interaction between S protein and the cellular receptor ACE2 (angiotensin converting enzyme-2). This complex is translocated to endosomes, S protein is cleaved by cathepsin L, membrane fusion occurs, and the viral genome is released. Viral proteins are transcribed from mRNAs, translated, nucleocapsids assembled in the cytoplasm (from genomic RNA and N protein), then processed through the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). The infected cell releases fully virulent, intact virions through exocytosis [18]. Courtesy of New York Blood Center/Dr. Shibo Jiang

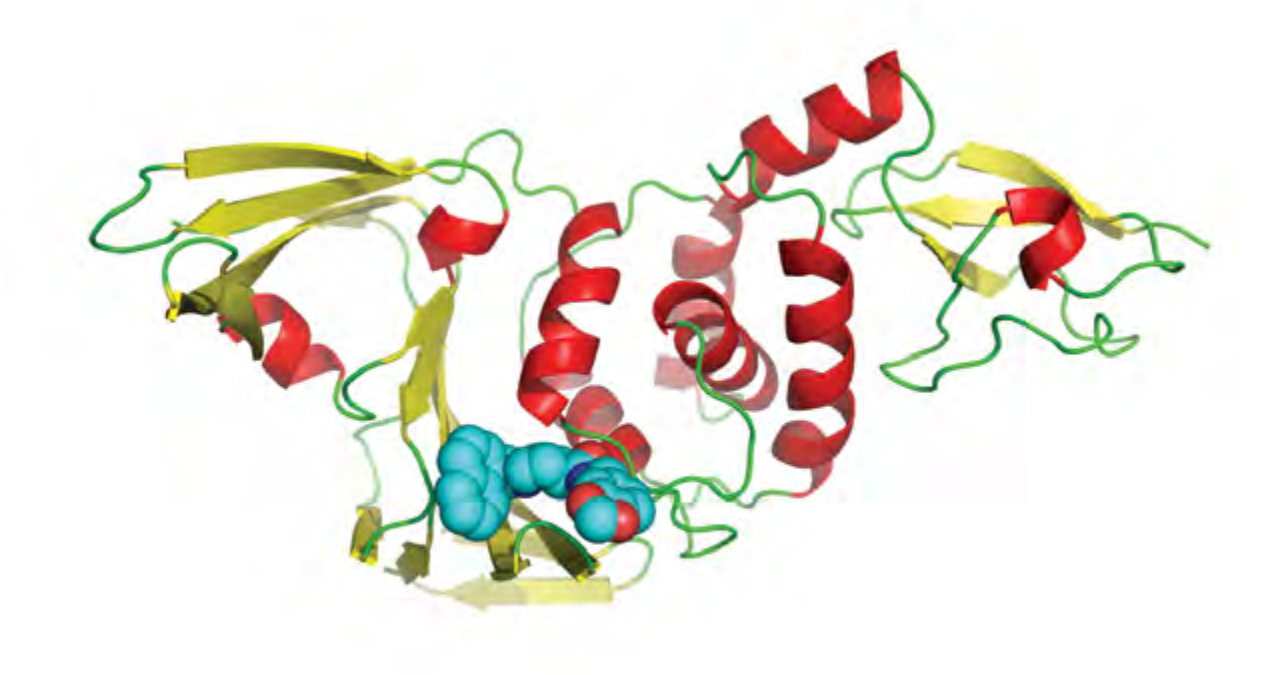
cathepsin L, and a secondary receptor that augments infection, L–SIGN, also was identified and characterized.

Researchers also have discovered that the Papain-like protease (PLpro) of SARS–CoV has deubiquitinating activity, which regulates the location and stability of cellular proteins. They also determined PLpro's three-dimensional structure [8], and this work is contributing to the design of small-molecule inhibitors of this essential enzyme (Figure 2).

Researchers at the Dale and Betty Bumpers Vaccine Research Center, part of NIAID, worked in partnership with Vical, Inc., to manufacture a candidate SARS vaccine that was found to prevent the SARS–CoV from replicating in laboratory mice. The vaccine, composed of a modified piece of DNA that encodes the S protein of SARS–CoV, is expected to stimulate protective immunity in humans. A Phase I open-label clinical study to evaluate safety, tolerability, and immune response to the vaccine was completed in December 2005. The study enrolled 10 healthy volunteers, aged 18 to 50 years, who were given a three-dose vaccine regimen at 1-month intervals. The vaccine was well tolerated, with no or mild systemic or local

FIGURE 2.

PLpro active site with inhibitor



The SARS–CoV papain-like protease (PLpro) enzyme is responsible for proteolytic processing of the viral polyprotein into its functional units. The PLpro active site is depicted in ribbon, and the noncovalent, lead inhibitor in space-filling (sphere) formats [19, 20]. Courtesy of Purdue University/Dr. Andrew D. Mesecar

reactogenicity and no serious adverse events. The vaccine induced neutralizing antibodies, which are strongly associated with recovery from natural SARS infection, and produced cellular immune responses that may be an important component of SARS immunity [9].

Other efforts have been taken by private industry to advance the development of a SARS vaccine. In May 2004, 36 volunteers in Beijing, China, received an inactivated SARS virus vaccine at two dosage levels. The candidate vaccine is produced by a Beijing-based company, Sinovac Biotech Ltd. Most volunteers receiving this vaccine generated an antibody response, and no obvious adverse side effects were noted [10].

Current State of the Science

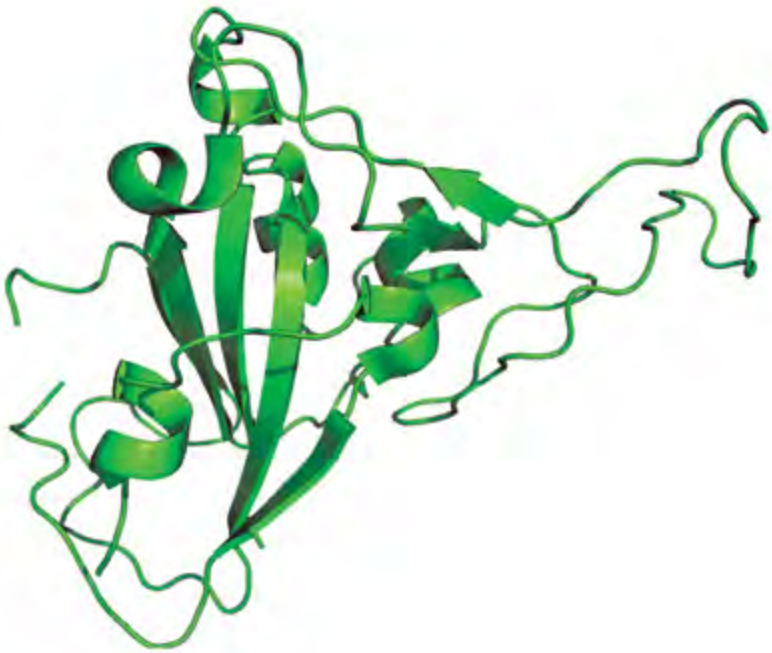
Because it is not known which type of vaccine will be most effective against SARS–CoV, NIAID supports several different approaches to vaccine development.

In 2003, NIAID awarded contracts for the production of experimental inactivated, whole-virus SARS vaccines as well as for the production of a recombinant S protein subunit vaccine [11, 12]. S protein is used by the virus to attach to lung cells. A contract also was awarded to support the generation of a monoclonal antibody to the S protein. This monoclonal antibody demonstrated both prophylactic and therapeutic properties in animals [13]. One of the contractors, Protein Sciences Corporation, has manufactured and released clinical-grade formulations of alum-adjuvanted and unadjuvanted recombinant baculovirus-produced SARS S protein [14]. An Investigational New Drug Application was submitted in mid-2011. The NIAID Vaccine and Treatment Evaluation Units [15] are planning to conduct a Phase I dose-escalation clinical trial of the candidate vaccine in 84 subjects.

In addition, NIAID-supported investigators are pursuing several other vaccine approaches: a soluble S protein SARS vaccine expressed from mammalian cells, an alphavirus replicon vaccine against SARS, and the expression of SARS proteins in virus-like particles. Two alternate strategies being developed are a peptide-based vaccine approach and an attenuated rhabdovirus (rabies) expressing the SARS S protein. As the vaccine development process is long and difficult, it is hoped that multiple strategies will prove safe and effective in animals and, ultimately, in humans.

FIGURE 3.

Receptor Binding Domain crystal structure



Depiction of the X-ray crystal structure of the SARS–CoV S protein receptor binding domain (RDB), amino acids 318–510, in ribbon format. The RDB is a promising subunit vaccine candidate for SARS–CoV [17, 18]. Courtesy of New York Blood Center/Dr. Shibo Jiang

Novel subunit vaccine constructs for an S protein SARS vaccine based on the receptor binding domain (RBD) are being developed by the New York Blood Center (Figure 3). Expression of S protein RBD constructs in 293T and CHO–K1 cells has been demonstrated. All RBD proteins expressed in different expression systems have high specificity and remain in intact conformation, as demonstrated by the binding of a panel of monoclonal antibodies. Recombinant RBD (rRBD) proteins made in various expression systems induce humoral immune responses, as demonstrated by the induction of high titers of antibodies that neutralize live SARS–CoV infection in vaccinated mice [16, 17].

In addition to the vaccine work described, considerable progress has been made on the development of therapeutics for SARS–CoV. Quantitative structure-activity relationship (QSAR) and other computational analysis provided input to further chemical improvement that resulted in a current lead inhibitor with an IC50 (half maximal inhibitory concentration) of 1.6 mM (millimolars) in an enzymatic assay and an EC50 (half maximal effective concentration) of 2.5 mM against the SARS virus in cell culture assays. The development of non-covalent PLpro inhibitors with micromolar antiviral activity appears significant. The crystal structure of PLpro complexed with a lead inhibitor provides a solid foundation for further design development. Investigators demonstrated the synergy in efficacy for 3C-like protease (3CLpro) and PLpro inhibitors, and they are now pursuing parallel discovery and development of therapeutic inhibitors of both the 3CLpro and PLpro enzymatic targets that appear to be most relevant to SARS [18, 19].

Alternative SARS–CoV inhibitors have been investigated based on their ability to block viral entry. Vinyl sulfides identified as very efficient inhibitors include K777, which previously was identified as an inhibitor of *Trypanosoma cruzi*. Second-generation analogs were generated and found to be between twofold and tenfold more potent than K777 and potent against other viruses as well, including Ebola and other human CoVs. Mannose-binding lectin (MBL) can directly inhibit SARS–CoV entry. Using a panel of spike mutants, an *N*-linked glycosylation close to the receptor binding site has been identified as the primary moiety involved in MBL binding, which demonstrated that MBL can inhibit entry only if applied prior to

cathepsin L activation [20]. Unlike several other viral envelopes to which MBL can bind, both recombinant and plasma-derived human MBL directly inhibited SARS–CoV-mediated viral infection. Mutagenesis indicated that a single *N*-linked glycosylation site, N330, was critical for the specific interactions between MBL and SARS–S. Despite the proximity of N330 to the receptor-binding motif of SARS–S, MBL did not affect interactions with the ACE2 receptor or cathepsin L-mediated activation of SARS–S-driven membrane fusion. Thus, binding of MBL to SARS–S may interfere with other early pre- or post-receptor binding events necessary for efficient virus entry [21].

In addition, NIAID contractors have screened 102,000 potential antiviral drugs and other compounds for activity against SARS–CoV. Several compounds have demonstrated antiviral activity and are being further tested in animal models.

Studies also have been conducted on the molecular mechanisms regulating SARS–CoV pathogenesis in young and aged mice. The resulting data suggest that the magnitude and kinetics of a disproportionately strong host innate immune response contributed to severe respiratory distress and lethality. Although the molecular mechanisms governing acute respiratory distress syndrome (ARDS) pathophysiology remain unknown in aged animals, these studies reveal a strategy for dissecting the genetic pathways by which SARS–CoV infection induces changes in the host response, leading to death [22]. The efficacies of candidate vaccines based on a Venezuelan equine encephalitits virus (VEE) attenuated viral replicon particles (VRP) bearing either attenuated (VRP(3014)) or wild-type VEE glycoproteins (VRP(3000)) were compared in young and aged mice. Aged animals receiving VRP(3000)-based vaccines were protected from SARS–CoV disease, while animals receiving the VRP(3014)-based vaccines were not. Because the glycoproteins of VRP(3014) strain differ from those of the wild-type virus by only three amino acids, tools are likely available to elucidate the mechanism of SARS–CoV protection in aged mice [23].

Researchers in NIAID's Laboratory of Infectious Diseases (LID) studied the replication of SARS–CoV in mice, hamsters, and nonhuman primates (NHPs) and established that intranasally administered SARS–CoV replicated efficiently in respiratory tissues. In BALB/c mice and hamsters, the virus replicated to levels that permit an evaluation of vaccines, immunotherapies, and antiviral drugs. In addition, further studies in mice and hamsters demonstrated that primary infection provides protection from re-infection and that antibodies alone can protect against viral replication. This work suggests that vaccines that induce neutralizing antibodies as well as strategies for immunoprophylaxis or immunotherapy are likely to be effective in combating SARS. LID scientists have collaborated with scientists at academic institutions to demonstrate the efficacy of monoclonal antibodies against the spike protein of SARS–CoV in preventing and treating SARS-associated disease in hamsters [13].

The LID investigators observed no clinical illness in young mice, hamsters, or NHPs infected with SARS–CoV. However, because advanced age has been associated with poorer outcome and greater mortality in SARS patients, the NIAID investigators examined whether aged mice might be susceptible to disease. They found that SARS–CoV-infected aged mice demonstrated signs of clinical illness that resolved by day 7 post-infection. The virus-infected aged mice mounted an adaptive immune response to infection; however, in contrast to young mice, they also mounted a proinflammatory cytokine response early post-infection. This work demonstrated in animals an age-related susceptibility to SARS that parallels the human experience [24]. The role of T cells in the pathogenesis and clearance of SARS–CoV was also evaluated in aged mice. Depletion of CD8+ T cells at the time of infection did not affect viral replication or clearance, but depletion of CD4+ T cells resulted in delayed clearance of SARS–CoV from the lungs and was associated with an enhanced immune-mediated interstitial pneumonitis. CD4+ T-cell depletion resulted in reduced neutralizing antibody and cytokine production and reduced pulmonary recruitment of inflammatory cells. Viral clearance in the absence of both CD4+ and CD8+ T cells and antibodies was associated with an innate immune response. These findings provide new insights into the role of CD4+ (but not CD8+ T cells) in primary SARS–CoV infection in this model [25].

The virus-host interactions that governed development of the acute end-stage lung disease cases and deaths from SARS are unknown. LID scientists collaborated with scientists at the University of North Carolina to demonstrate that in mice, SARS–CoV pathogenesis is regulated by a STAT1-dependent but type I, II, and III interferon-independent mechanism. These scientists propose that STAT1 primarily protects mice via its role as an antagonist of unrestrained cell proliferation [26].

The LID scientists also have collaborated with other scientists at the National Institutes of Health, as well as researchers at academic institutions and in industry, to evaluate a number of candidate SARS–CoV vaccines, including inactivated, subunit, vectored, and DNA vaccines, in animal models.

Challenges and Opportunities

The re-emergence of SARS is possible, and the need remains for commercial vaccine and therapeutic development. However, the cost and length of time for product development, and the uncertain future demand, result in unfavorable economic conditions to accomplish this task.

A better understanding of the abilities of and requirements for the SARS virus to infect animals without detrimental effect, and to pass from animal to animal (horseshoe bat to civet) as well as from animal to human, is needed. Findings from this research also could apply to the many other viruses that pass from animals to humans [27].

The potential exists for the exacerbation of disease on exposure to those who have been immunized, as has been seen with respiratory syncytial virus, dengue virus, and feline infectious peritonitis virus [28]. Animal studies suggest that this immunopotentiation may occur with candidate SARS–CoV vaccines that contain the N protein [29].

Improved small- and large-animal models for SARS are needed, particularly those models that better mimic human disease with respect to clinical course and symptoms. Improved animal models will help illuminate the pathophysiology of disease, including innate and adaptive immune responses and immunopotentiation, and help move vaccines and therapeutics through the regulatory and clinical phases and ultimately to licensure [30].

In the development of therapeutics and next-generation vaccines, more work is required to determine the structure/function relationships of critical enzymes and structural proteins. Once these relationships are better understood, improvements to the design of small-molecule and protein inhibitors can occur.

A long-term public health strategy should include both active and passive SARS vaccines as well as therapeutics. This strategy should focus on the impact of the disease on healthcare and service workers and on the elderly, as well as mitigation of economic impact.

As the first pandemic of the 21st century, SARS has provided a unique opportunity for research on the life cycle and components of an emerging or re-emerging disease. Although further research is needed, many recent accomplishments are leading the way toward the development of effective prevention and treatment measures.

REFERENCES

1. World Health Organization. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003 [Global Alert and Response on the Internet]. Geneva: World Health Organization; 2003 Dec 31. Accessed from: www.who.int/csr/sars/country/table2004_04_21/en/index.html
2. Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YS, et al. The genome sequence of the SARS-associated coronavirus. Science. 2003 May 30;300(5624):1399-404. Epub 2003 May 1.
3. World Health Organization. Update 49—SARS case fatality ratio, incubation period [Global Alert and Response on the Internet]. Geneva: World Health Organization; 2003 May 7. Accessed from: www.who.int/csr/sarsarchive/2003_05_07a/en/
4. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, et al. Bats are natural reservoirs of SARS-like coronaviruses. Science. 2005 Oct 28;310(5748):676-9. Epub 2005 Sep 29.
5. Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci U S A. 2005 Sep 27;102(39):14040-5. Epub 2005 Sep 16.
6. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003 Nov 27;426(6965):450-4.
7. Li W, Wong SK, Li F, Kuhn JH, Huang IC, Choe H, et al. Animal origins of the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein interactions. J Virol. 2006 May;80(9):4211-9.
8. Ratia K, Saikatendu KS, Santarsiero BD, Barretto N, Baker SC, Stevens RC, et al. Severe acute respiratory syndrome coronavirus papain-like protease: structure of a viral deubiquitinating enzyme. Proc Natl Acad Sci U S A. 2006 Apr 11;103(15):5717-22. Epub 2006 Mar 31.
9. Martin JE, Louder MK, Holman LA, Gordon IJ, Enama ME, Larkin BD, et al; VRC 301 Study Team. A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial. Vaccine. 2008 Nov 25;26(50):6338-43. Epub 2008 Sep 26.
10. Lin JT, Zhang JS, Su N, Xu JG, Wang N, Chen JT, et al. Safety and immunogenicity from a Phase I trial of inactivated severe acute respiratory syndrome coronavirus vaccine. Antivir Ther. 2007;12(7):1107-13.
11. Spruth M, Kistner O, Savidis-Dacho H, Hitter E, Crowe B, Gerencer M, et al. A double-inactivated whole virus candidate SARS coronavirus vaccine stimulates neutralising and protective antibody responses. Vaccine. 2006 Jan 30;24(5):652-61. Epub 2005 Aug 26.
12. Kusters I, Matthews J, Saluzzo JF. Manufacturing vaccines for an emerging viral infection—specific issues associated with the development of a prototype SARS vaccine. In: Barrett A, Stanberry L, editors. Vaccines for biodefense and emerging and neglected diseases. London: Academic Press; 2009. p. 148-57.
13. Roberts A, Thomas WD, Guarner J, Lamirande EW, Babcock GJ, Greenough TC, et al. Therapy with a severe acute respiratory syndrome-associated coronavirus-neutralizing human monoclonal antibody reduces disease severity and viral burden in golden Syrian hamsters. J Infect Dis. 2006 Mar 1;193(5):685-92. Epub 2006 Jan 27.

14. Zhou Z, Post P, Chubet R, Holtz K, McPherson C, Petric M, et al. A recombinant baculovirus-expressed S glycoprotein vaccine elicits high titers of SARS-associated coronavirus (SARS–CoV) neutralizing antibodies in mice. Vaccine. 2006 Apr 24;24(17):3624-31. Epub 2006 Feb 9.

15. National Institutes of Allergy and Infectious Diseases. Vaccine and treatment evaluation units (VTEUs) [Internet]. Bethesda (MD): National Institutes of Allergy and Infectious Diseases; c2010. Accessed from: www.niaid.nih.gov/about/organization/dmid/clinical/vteu/Pages/default.aspx

16. Du L, Zhao G, Chan CC, Sun S, Chen M, Liu Z, et al. Recombinant receptor-binding domain of SARS–CoV spike protein expressed in mammalian, insect and *E. coli* cells elicits potent neutralizing antibody and protective immunity. Virology. 2009 Oct 10;393(1):144-50. Epub 2009 Aug 15.

17. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS–CoV—a target for vaccine and therapeutic development. Nat Rev Microbiol. 2009 Mar;7(3):226-36. Epub 2009 Feb 9.

18. Ratia K, Pegan S, Takayama J, Sleeman K, Coughlin M, Baliji S, et al. A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. Proc Natl Acad Sci U S A. 2008 Oct 21;105(42):16119-24. Epub 2008 Oct 13.

19. Ghosh AK, Takayama J, Aubin Y, Ratia K, Chaudhuri R, Baez Y, et al. Structure-based design, synthesis, and biological evaluation of a series of novel and reversible inhibitors for the severe acute respiratory syndrome-coronavirus papain-like protease. J Med Chem. 2009 Aug 27;52(16):5228-40.

20. Glowacka I, Bertram S, Herzog P, Pfefferle S, Steffen I, Muench MO, et al. Differential downregulation of ACE2 by the spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus NL63. J Virol. 2010 Jan;84(2):1198-205. Epub 2009 Oct 28.

21. Zhou Y, Lu K, Pfefferle S, Bertram S, Glowacka I, Drosten C, et al. A single asparagine-linked glycosylation site of the severe acute respiratory syndrome coronavirus spike glycoprotein facilitates inhibition by mannose-binding lectin through multiple mechanisms. J Virol. 2010 Sep;84(17):8753-64. Epub 2010 Jun 23.

22. Rockx B, Baas T, Zornetzer GA, Haagmans B, Sheahan T, Frieman M, et al. Early upregulation of acute respiratory distress syndrome-associated cytokines promotes lethal disease in an aged-mouse model of severe acute respiratory syndrome coronavirus infection. J Virol. 2009 Jul;83(14):7062-74. Epub 2009 May 6.

23. Sheahan T, Whitmore A, Long K, Ferris M, Rockx B, Funkhouser W, et al. Successful vaccination strategies that protect aged mice from lethal challenge from influenza virus and heterologous severe acute respiratory syndrome coronavirus. J Virol. 2011 Jan;85(1):217-30. Epub 2010 Oct 27.

24. Roberts A, Vogel L, Guarner J, Hayes N, Murphy B, Zaki S, et al. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. J Virol. 2005 Jan;79(1):503-11.

25. Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, et al. Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS–CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS–CoV infection. J Virol. 2010 Feb;84(3):1289-301. Epub 2009 Nov 11.

26. Frieman MB, Chen J, Morrison TE, Whitmore A, Funkhouser W, Ward JM, et al. SARS–CoV pathogenesis is regulated by a STAT1 dependent but a type I, II and III interferon receptor independent mechanism. PLoS Pathog. 2010 Apr 8;6(4):e1000849.

27. Graham RL, Baric RS. Recombination, reservoirs, and the modular spike: mechanisms of coronavirus cross-species transmission. J Virol. 2010 Apr;84(7):3134-46. Epub 2009 Nov 11.

28. Perlman S, Dandekar AA. Immunopathogenesis of coronavirus infections: implications for SARS. Nat Rev Immunol. 2005 Dec;5(12):917-27.

29. Deming D, Sheahan T, Heise M, Yount B, Davis N, Sims A, et al. Vaccine efficacy in senescent mice challenged with recombinant SARS–CoV bearing epidemic and zoonotic spike variants. PLoS Med. 2006 Dec;3(12):e525.

30. Subbarao K, Roberts A. Is there an ideal animal model for SARS? Trends Microbiol. 2006 Jul;14(7):299-303. Epub 2006 Jun 8.

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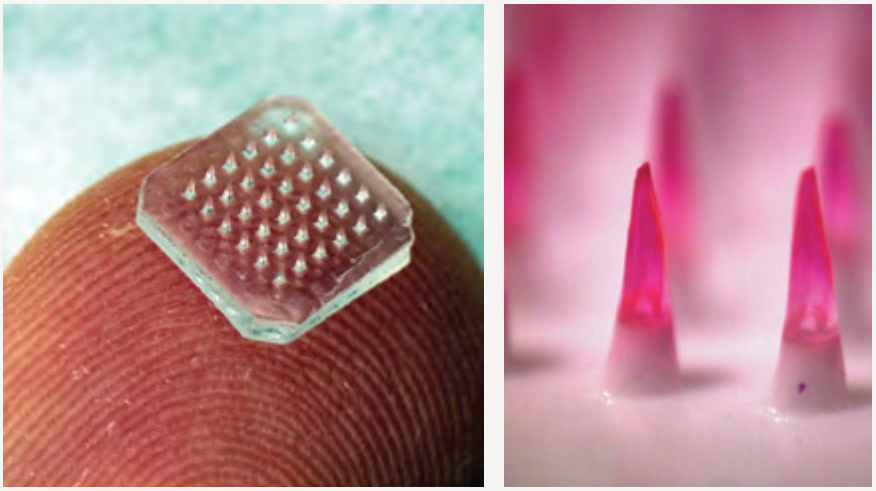
Vaccines offer the most effective method of protecting the public against infectious diseases. However, most currently licensed vaccines require multiple doses to achieve immunity, and each vaccine has unique storage requirements and different methods of administration. New and improved vaccines must be safe and easy to administer and must rapidly produce a protective immune response. Vaccines also must be safe and efficacious in populations of varying age and health status. To prepare for epidemic outbreaks of infectious diseases or the intentional release of biothreat pathogens, improved product stability and vaccination effectiveness are of great importance. Novel delivery technologies that are simple and effective could potentially help a variety of vaccines fulfill these requirements and also could have a major impact on worldwide vaccination campaigns.

New vaccine delivery technologies have evolved as we have increased our understanding of the biology of diseases and the immune response needed to confer protection. The first delivery technologies used needles (smallpox) or needles and syringes (diphtheria, pertussis, tetanus) to deliver vaccines through the surface of the skin. The next innovation was an oral vaccine (polio), and most recently, an intranasal vaccine (influenza) has been developed. Advances in biochemistry and molecular biology have enabled vaccine developers to manufacture greater amounts of vaccines with greater purity, which results in reduced costs and increased product safety. Similarly, formulation technologies have been discovered that enhance the ability of vaccines to produce protective immune responses and stabilize vaccines for storage and use in new delivery systems.



LEFT: A patch containing 36 dissolving microneedles is shown on a fingertip. Courtesy of Georgia Institute of Technology/Jeong-Woo Lee; RIGHT: Microscope image shows dissolving microneedles encapsulating a pink dye. The microneedles dissolve wihin minutes after inserstion into skin to release encapsulated drug or vaccine. Courtesy of Georgia Institute of Technology/Sean Sullivan

Recently developed vaccines, while still delivered with a needle and syringe, are quite different from vaccines of the past. Dose volumes are decreased due to increased purity, and new adjuvants are being used to help trigger the desired immune responses. Novel methods under evaluation to deliver vaccine through the skin include vaccine-coated microneedles, very small needles that contain the vaccine and are dissolved by the body's fluids just below the skin. Some DNA vaccine developers have been testing the feasibility of using electric current to carry their vaccines through the skin. Currently also in the testing stages are a group of small hand-held "needle free" devices that generate jets of high pressure air to "inject" the vaccine through the skin.

Oral delivery offers the advantage of ease of administration, while presenting unique challenges to vaccine developers. Vaccines must be able to survive the varying chemical and microbiological environments of the digestive tract and still be able to elicit the desired immune response. Orally delivered modified live bacterial (typhoid) and viral (polio, rotavirus) vaccines have been successful.

Intranasal delivery of vaccines has been investigated for a number of years with some success. This route has been tested with vaccines delivered in mists, powders, and emulsions. Unique formulations must be designed to enable vaccines to reach immune processing cells located in the nostrils. Challenges of intranasal delivery include the possibility of expelling the vaccine from the nose by an involuntary sneezing reflex, swallowing the vaccine if it is not retained in the nostrils, or inhaling the vaccine into the lungs. Any of these events can negate the vaccine's utility.

As new scientific discoveries are used to improve manufacturing, formulation, and delivery technologies of vaccines, the worldwide population will benefit from reduced time to protective immunity, increased vaccine stability, and reduced logistical requirements for storage, transportation, and delivery.

West Nile Virus

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The identification of West Nile virus (WNV) in New York in the summer of 1999 was the first time the mosquito-borne microbe had been detected in the Western Hemisphere. Until then, the virus had been found chiefly in Africa, Eastern Europe, the Middle East, and Asia. Since 1999, WNV has spread throughout the continental United States; as of October 25, 2011, 555 cases in 42 states and the District of Columbia have been confirmed by the Centers for Disease Control and Prevention (CDC) [1]. Although infection with WNV is usually asymptomatic or causes only mild symptoms in humans, it can spread to the central nervous system and cause a variety of disease outcomes, including encephalitis, a potentially deadly brain inflammation. Other clinical presentations can be similar to those of Parkinson's disease, poliomyelitis, or Alzheimer's disease. Most cases of West Nile neurologic disease occur in elderly people and in those with impaired immune systems (people with diabetes, chemotherapy patients, etc.) [2,3]. The realization in 2002 that WNV can be transmitted by blood transfusion or organ transplantation from WNV-infected donors prompted stringent safety testing of donor blood supplies [4]. Many published studies of patients with WNV meningitis or encephalitis have confirmed that those older than 55 years are more likely to have a lengthy recovery period with long-term physical, cognitive, and functional disabilities that may last more than 2 years after acute illness [5]. Despite much effort over the last decade to develop vaccines and therapeutics, no treatment is available for WNV encephalitis, and no licensed vaccine exists to prevent disease in humans. (Although WNV vaccines have been available for prevention of disease in horses since 2002, development of vaccines for human use must adhere to Food and Drug Administration (FDA)-mandated stringent safety and efficacy testing, which extends the development timeline.) Mosquito control measures and other tactics, such as the use of mosquito repellents and the wearing of long-sleeved shirts and pants to reduce the number of mosquito bites, have thus been the only available strategies to combat the rapid spread of this emerging disease.

Faced with the continued potential for a serious WNV epidemic, researchers supported by the National Institute of Allergy and Infectious Diseases (NIAID) initiated development of candidate vaccines to protect against WNV infection. WNV vaccine development has benefited from the fact that the virus belongs to a taxonomic group known as flaviviruses, which share a number of characteristics that allow scientists to build on earlier discoveries about other flaviviruses that are closely related to WNV, including Japanese encephalitis virus, St. Louis encephalitis virus, yellow fever virus, and dengue virus.

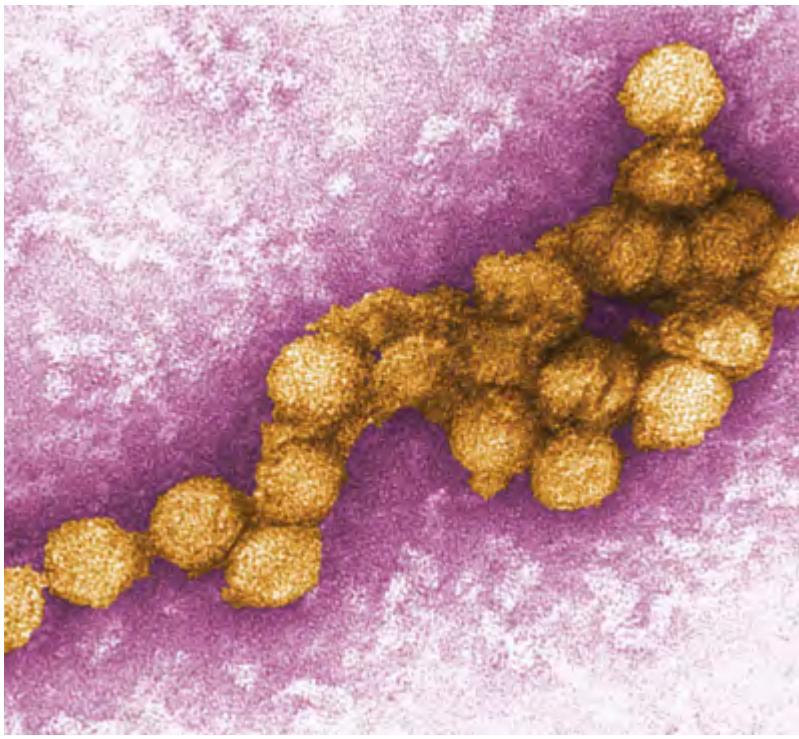
There has been great success in controlling yellow fever and Japanese encephalitis with well-organized vaccination campaigns centered on efficacious vaccines [6]. Therefore, the National Institutes of Health (NIH) has encouraged similar WNV vaccine development programs.

NIAID-supported basic research studies discovered that hamsters and mice are good models for WNV disease in humans. NIAID-supported researchers at the University of Texas Medical Branch, Galveston, conducted a series of preliminary experiments to learn more precisely the degree of protection that candidate WNV and other licensed flavivirus vaccines might have against WNV. Researchers found that golden hamsters were completely protected by prototype WNV vaccines and, surprisingly, were also at least partially protected against WNV infection by licensed Japanese encephalitis and yellow fever vaccines [7]. Thus, this animal model is an important resource that now is being used to test the efficacy of new vaccine candidates and antiviral medicines. Similarly, efficacious mouse models of WNV encephalitis also have been developed with NIAID support [8].

NIAID is supporting a number of WNV vaccine approaches. One of the earliest began in 1999 when NIAID funded a fast-track project by Acambis, Inc., to develop a candidate live, attenuated, "chimeric" WNV vaccine. The vaccine was constructed using the DNA/genes of the licensed yellow fever 17D vaccine virus as the backbone. For the WNV vaccine, researchers substituted certain genes (the premembrane (prM) and envelope (E) surface protein genes) of WNV for the prM and E genes of the yellow fever vaccine virus using chimeric technology that was originally developed at NIAID during the early 1990s. The "chimeric" yellow fever/West Nile DNA was then manipulated and inoculated into cell cultures

to produce the "chimeric" West Nile live, attenuated vaccine that was able to elicit anti-WNV antibodies and protect against WNV infection in vaccinated animals. This method of creating chimeric flavivirus vaccines is also being applied to developing vaccines for dengue and Japanese encephalitis viruses. The Acambis WNV vaccine (designated ChimeriVaxWN) has undergone successful preclinical evaluations in hamsters, mice, monkeys, and horses and yielded encouraging results in a Phase I clinical trial [9]. In December 2005, the vaccine was moved into Phase II clinical trial evaluation, making Acambis the first company to enter Phase II testing of a WNV vaccine. The randomized, double-blind, placebo-controlled trial was conducted in more than 200 subjects in the United States. The safety, tolerability, and immunogenicity of the vaccine at different dose levels was evaluated in a two-part study, first in healthy young adults aged 18–40 years, then in two healthy, elderly range cohorts, aged 41–64 years and age >65 years. The recently published results showed the vaccine to be highly immunogenic and well-tolerated at all dose levels and in all age groups studied. The incidence and severity of treatment-emergent adverse events (primarily fatigue, headache, and myalagia) were comparable between placebo groups and all treatment groups [10]. In 2008, Sanofi Pasteur acquired Acambis, and Acambis' West Nile, dengue, and Japanese encephalitis candidate vaccine products are now integrated within the Sanofi Pasteur vaccine development schedule.

Intramural NIAID scientists, with early assistance from collaborators from the Walter Reed Army Institute of Research (WRAIR), capitalized on advances in recombinant DNA technology and previous research on dengue viruses to produce a different candidate live, attenuated WNV vaccine. The NIAID team already had successfully tested a strategy that used the new technology to replace key genes of different flaviviruses with those of dengue virus type 4 (DENV–4). DENV–4 is a non-neuroinvasive virus that does not cause neurological disease in animals and humans infected peripherally. The resulting weakened, or attenuated, virus strains were safer for use in a vaccine, but still protective. The NIAID team then used this strategy to combine genes from WNV and DENV–4. This hybrid virus did not infect the brain, yet still stimulated a strong immune response with even a single dose. This WNV/DENV–4 chimeric virus was further attenuated for mice and monkeys by deleting 30 nucleotides from its 3' untranslated region (designated delta30) [11]. The WNV/DENV–4 3'delta30 candidate vaccine was evaluated for safety and immunogenicity in a Phase I clinical trial that is now completed.



Transmission electron micrograph (TEM) of the West Nile virus (WNV). Courtesy of CDC

NIAID scientists at the Dale and Betty Bumpers Vaccine Research Center (VRC) developed a DNA-based vaccine against WNV in collaboration with the CDC and the San Diego-based biotechnology company Vical, Inc. The vaccine is based on an existing codon modified gene-based DNA plasmid vaccine platform designed to express WNV proteins. Two versions of the vaccine were developed, one utilizing an optimized CMV/R promoter. The VRC has completed two Phase I clinical trials to evaluate safety, tolerability, and immune responses of these recombinant DNA vaccines in human volunteers [12, 13]. As the DNA vaccine has been licensed to Vical by the CDC, any further development will be undertaken by Vical.

In addition to pursuing replicating chimeric vaccines, researchers have made advances in the development of nonreplicating subunit vaccines. Scientists at Hawaii Biotech, Inc., supported initially by an NIAID grant and then by a National Institute of Neurological Disorders and Stroke (NINDS) grant, along with other financing, are developing genetically engineered, *Drosophila*-expressed subunit vaccines containing portions of the viral E and NS1 proteins. Subunit protein vaccines cannot replicate or cause disease. Following testing of the company's WNV vaccine in the golden hamster and nonhuman primate WNV disease models [14], the WNV vaccine (designated HBV–002) completed a successful Phase

I clinical trial in 2008, which demonstrated its safety and immunogenicity in healthy adult volunteers. The company is planning future clinical trials in other populations (e.g., elderly, immunocompromised).

At L2 Diagnostics, LLC, NIAID-supported researchers have developed a recombinant *Baculovirus*-produced subunit vaccine that induces protective antiviral antibodies in a murine model of WNV infection and, importantly, prevents WNV disease in horses [15]. No Phase I clinical trials are yet planned; however, the company may pursue regulatory approvals for veterinary use of this vaccine. The company is also investigating a nanoparticle vaccine against WNV.

Other WNV vaccines in early-stage development include a mutagenized live, attenuated vaccine based on Kunjin virus (an Australian strain of WNV that is closely related to the WNV NY99 strain but rarely associated with clinical disease), a novel live attenuated vaccine (RepliVax WN) composed of WNV particles that are limited to a single cycle of replication that limits spread and renders it incapable of causing disease [16], a proprietary inactivated vaccine formulation, a dry powder WNV protein vaccine that could be administered intranasally, and a synthetic peptide-based multi-flavivirus vaccine.

REFERENCES

1. Centers for Disease Control and Prevention (CDC). 2011 West Nile virus human infections in the United States (reported to CDC as of October 25, 2011) [Internet]. Atlanta (GA): CDC; 2011. Available from: www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount11_detailed.htm
2. Davis LE, DeBiasi R, Goade DE, Haaland KY, Harrington JA, Harnar JB, et al. West Nile virus neuroinvasive disease. Ann Neurol. 2006 Sep;60(3):286-300.
3. Murray KO, Walker C, Gould E. The virology, epidemiology, and clinical impact of West Nile virus: a decade of advancements in research since its introduction into the Western Hemisphere. Epidemiol Infect. 2011 Jun;139(6):807-17. Epub 2011 Feb 23.
4. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, et al. Screening the blood supply for West Nile virus RNA by nucleic acid amplification testing. N Engl J Med. 2005 Aug 4;353(5):460-7.
5. Sejvar, JJ. The long-term outcomes of human West Nile virus infection. Clin Infect Dis. 2007 Jun 15;44(12):1617-24. Epub 2007 May 2.
6. Chang GJ, Kuno G, Purdy DE, Davis BS. Recent advancement in flavivirus vaccine development. Expert Rev Vaccines. 2004 Apr;3(2):199-220.
7. Tesh RB, Travassos da Rosa AP, Guzman H, Araujo TP, Xiao SY. Immunization with heterologous flaviviruses protective against fatal West Nile encephalitis. Emerg Infect Dis. 2002 Mar;8(3):245-51.
8. Brown AN, Kent KA, Bennett CJ, Bernard KA. Tissue tropism and neuroinvasion of West Nile virus do not differ for two mouse strains with different survival rates. Virology. 2007 Nov 25;368(2):422-30. Epub 2007 Aug 6.
9. Monath TP, Liu J, Kanesa-Thasan N, Myers GA, Nichols R, Deary A, et al. A live, attenuated recombinant West Nile virus vaccine. Proc Natl Acad Sci U S A. 2006 Apr 25;103(17):6694-9. Epub 2006 Apr 14.
10. Biedenbender R, Bevilacqua J, Gregg AM, Watson M, Dayan G. Phase II, randomized, double-blind, placebo-controlled, multicenter study to investigate the immunogenicity and safety of a West Nile virus vaccine in healthy adults. J Infect Dis. 2011 Jan 1;203(1):75-84.
11. Pletnev AG, Swayne DE, Speicher J, Rumyantsev AA, Murphy BR. Chimeric West Nile/dengue virus vaccine candidate: preclinical evaluation in mice, geese and monkeys for safety and immunogenicity. Vaccine. 2006 Sep 29;24(40-41):6392-404. Epub 2006 Jun 21.
12. Martin JE, Pierson TC, Hubka S, Rucker S, Gordon IJ, Enama ME, et al. A West Nile virus DNA vaccine induces neutralizing antibody in healthy adults during a Phase I clinical trial. J Infect Dis. 2007 Dec 15;196(12):1732-40.
13. Ledgerwood JE, Pierson TC, Hubka SA, Desai N, Rucker S, Gordon IJ, et al; VRC 303 Study Team. A West Nile virus DNA vaccine utilizing a modified promoter induces neutralizing antibody in younger and older healthy adults in a Phase I clinical trial. J Infect Dis. 2011 May 15;203(10):1396-404. Epub 2011 Mar 11.
14. Lieberman MM, Nerurkar VR, Luo H, Cropp B, Carrion R Jr, de la Garza M, et al. Immunogenicity and protective efficacy of a recombinant subunit West Nile virus vaccine in rhesus monkeys. Clin Vaccine Immunol. 2009 Sep;16(9):1332-7. Epub 2009 Jul 29.
15. Bonafé N, Rininger JA, Chubet RG, Foellmer HG, Fader S, Anderson JF, et al. A recombinant West Nile virus envelope protein vaccine candidate produced in *Spodoptera frugiperda* expresSF+ cells. Vaccine. 2009 Jan 7;27(2):213-22. Epub 2008 Nov 7.
16. Nelson MH, Winkelmann E, Ma Y, Xia J, Mason PW, Bourne N, et al. Immunogenicity of RepliVAX WN, a novel single-cycle West Nile virus vaccine. Vaccine. 2010 Dec 16;29(2):174-82. Epub 2010 Nov 4.

HENIPAVIRUSES (NIPAH VIRUS AND HENDRA VIRUS)

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Nipah virus and Hendra virus are closely related paramyxoviruses that emerged from bats during the 1990s to cause deadly outbreaks in humans and domesticated animals [1]. Hendra virus was first discovered in 1994 in Australia, where it caused outbreaks in racing horses and horse handlers [2]. Fourteen outbreaks have occurred in Australia from 1994 to 2010, causing 7 human infections and 4 deaths [3, 4]. Hendra outbreaks have increased in frequency; between June and October 2011 alone there were 18 spillover events in horses and 1 dog, with no confirmed human infections [5, 6]. Queensland and New South Wales have now been declared endemic for Hendra virus. Field studies following the outbreaks identified large fruit bats (*Pteropus giganteus*) as the source of infection. These bats roost on trees in horse pastures, and it is believed that horses became infected by nibbling on leftover fruit eaten by the bats or by exposure to bat secretions found in the pasture. Nipah virus was first identified in 1998 after a large outbreak in pig farms in the Malaysian peninsula caused 265 human infections and 105 deaths [7]. This epidemic is believed to have started in pig farms built on the edge of a forest where large fruit bats were roosting. Nipah virus, which is carried by bats, was passed to pigs when the pigs fed on fruit contaminated with bat saliva, which the bats dropped from their roost into the pig enclosures [8]. The infected pigs developed severe respiratory and neurological disease and are believed to have infected humans through respiratory droplets. The Nipah outbreaks in Malaysia had a devastating effect on the economy, as more than 1 million pigs had to be culled, and 800 farms had to be demolished. Several additional outbreaks have occurred in parts of Bangladesh and India, with a human case fatality rate of approximately 70 percent. Some of these outbreaks have been linked to the human consumption of fresh palm sap [9]. Field investigations have shown that palm sap, which is collected from the bark of palm trees, is often contaminated with bat saliva, as the bats like to feed from the sap-collection vessels.

No vaccine or therapeutic agents are currently available to prevent or treat Hendra and Nipah infections.

National Institute of Allergy and Infectious Diseases (NIAID)-supported investigators developed vaccines for Nipah and Hendra virus based on the soluble G-glycoproteins of the viruses formulated with adjuvants. Both vaccines have been shown to induce strong neutralizing antibodies in different laboratory animals [10, 11]. Importantly, the Hendra virus vaccine induces cross-neutralizing antibodies against Nipah virus. The Hendra virus vaccine has been shown to confer 100 percent protection against lethal viral challenges with both Nipah and Hendra viruses in cats, ferrets, and nonhuman primates [10, 11]. In May 2011, scientists at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia announced that this vaccine protected horses from lethal challenges with Hendra virus. In late 2011, Pfizer licensed the technology to make the vaccine for veterinary use. This vaccine has the potential to protect domesticated animals from infection and stop animal-to-human transmission of Nipah and Hendra viruses in endemic countries.

REFERENCES

1. Field HE, MacKenzie JS, Daszak P. Henipaviruses: emerging paramyxoviruses associated with fruit bats. Curr Top Microbiol Immunol. 2007;(315):133-59.
2. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. Nat Rev Microbiol. 2006 Jan;4(1):23-35.
3. Playford EG, McCall B, Smith G, Slinko V, Allen G, Smith I, et al. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. Emerg Infect Dis. 2010 Feb;16(2):219-23.
4. Hendra virus, equine—Australia (03): Queensland, human exposure. In: ProMED-mail (www.promedmail.org, archive no. 20090821.2963) [Internet]. Brookline (MA): International Society for Infectious Diseases; 2009.
5. Hendra virus, equine—Australia (18): (Queensland) canine. In: ProMED-mail (www.promedmail.org, archive no. 20110727.2257) [Internet]. Brookline (MA): International Society for Infectious Diseases; 2011.
6. Hendra virus, equine—Australia (28): Queensland, New South Wales. ProMED-mail (www.promedmail.org, archive no. 20111013.3061) [Internet]. Brookline (MA): International Society for Infectious Diseases; 2011.
7. Chua KB. Nipah virus outbreak in Malaysia. J Clin Virol. 2003 Apr;26(3):265-75.
8. Field HE. Bats and emerging zoonoses: henipaviruses and SARS. Zoonoses Public Health. 2009 Aug;56(6-7):278-84.
9. Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, Gurley E, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis. 2006 Dec;12(12):1888-94.
10. Pallister J, Middleton D, Wang LF, Klein R, Haining J, Robinson R, et al. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. Vaccine. 2011 Aug 5;29(34):5623-30. Epub 2011 Jul 1.
11. McEachern JA, Bingham J, Crameri G, Green DJ, Hancock TJ, Middleton D, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine, 2008 Jul 23;26(31):3842-52.

Group B Streptococcus

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In the 1970s, group B streptococcus (GBS) emerged as the leading infectious cause of neonatal morbidity and mortality and late pregnancy-related morbidity [1, 2]. Two syndromes in neonates and young infants were recognized: early-onset disease (primarily sepsis, pneumonia, and meningitis within the first 6 days of life) and late-onset disease (primarily meningitis between 7 and 90 days of age). GBS bacteria are vertically transferred from a vaginally or rectally colonized mother to the neonate during labor and delivery, also called the intrapartum period. This typically results in colonization of the infant and less commonly in invasive early-onset disease. The mode of transmission for late-onset disease remains poorly elucidated.

Neonatal disease prevention strategies in the United States have focused on antenatal identification of GBS vaginal and rectal colonization in pregnant women and the use of antibiotics during labor and delivery in women who are colonized or at risk of colonization. This has led to an 80 percent decrease in the incidence of early-onset neonatal infections and a 21 percent decrease in the incidence of invasive GBS infections in pregnant women, associated with intrapartum antibiotic prophylaxis [3, 4]. Although the maternal intrapartum chemoprophylaxis strategy is effective, it is an interim solution, as the incidence of late-onset GBS disease remains unchanged. Additionally, chemoprophylaxis has resulted in use of antibiotics in 30 percent of women at delivery, raising concerns about the emergence of resistant strains [5, 6]. Recent data indicate that 20 percent of GBS isolates are resistant to clindamycin and 30–40 percent are resistant to erythromycin. Fortunately, penicillin resistance is not yet an issue [7, 8].

During the last two decades, an increase in the incidence of invasive GBS disease in nonpregnant adults has been reported [1, 9]. The majority of these cases occur in adults with underlying medical conditions, such as diabetes, neurological impairment, breast cancer, and cirrhosis, but the highest attack rates occur in those aged 65 years and older. Common clinical manifestations of GBS disease in adults include skin and soft tissue infections, bacteremia and sepsis, bone and joint infections, and pneumonia. Meningitis and endocarditis are less common, but are associated with serious morbidity and mortality. The case fatality rate is higher in adults than in neonates, and is especially high in those over the age of 65.

A safe and effective vaccine would be a major advance in the prevention of GBS disease. Active immunization of women during the third trimester of pregnancy has potential for the prevention of both maternal and infant GBS disease. Adults with underlying medical conditions also could benefit significantly from a GBS vaccine. A licensed vaccine is not yet available, but several promising vaccine candidates are in early stages of development.

Since the early 1990s, contracts funded by the National Institute of Allergy and Infectious Diseases (NIAID) have supported GBS vaccine design studies, the production of GBS glycoconjugate vaccines for serotypes Ia, Ib, II, III, and V, and more than 20 NIAID-sponsored Phase I and Phase II trials. In these studies, study participants received uncoupled capsular polysaccharides (CPSs) or CPS-protein conjugates. Each CPS was individually conjugated to tetanus toxoid (TT) or the mutant diphtheria toxoid cross-reactive material 197 (CRM197) [10–13]. In summary, results indicated that the conjugate vaccines were safe and induced functional antibody responses.

Most clinical trials involved a single injection of monovalent vaccine preparations, with the exception of a bivalent study in which type II–TT and type III–TT were administered together [14]. The immune response in bivalent vaccine recipients was comparable to that observed in the monovalent vaccine recipients. One study, in which volunteers received a type III–TT booster 21 months after the first dose, revealed that a booster response was only observed in a group that had undetectable GBS type III CPS-specific immunoglobulin G (IgG) before the first dose of type III–TT vaccine [15]. Another study showed that adsorption of a type III–TT to alum did not improve the immune response, compared with the type III CPS [15]. A randomized, double-blind, Phase I study was completed in which a GBS type III–TT was administered to 30 healthy, third-trimester pregnant women [16]. The vaccine was safe, healthy babies were delivered by all vaccine recipients, and vaccine-induced type III CPS-specific IgG was shown to be efficiently transported to the infant and functionally active through 2 months of age. These data suggest that a GBS conjugate

vaccine has the potential to prevent both early- and late-onset infant GBS disease and invasive disease in pregnant women.

More recently, additional studies have been conducted and are summarized below.

- A randomized, double-blind comparison study with GBS type V–TT and GBS type V–CRM197 vaccines tested in 35 healthy, nonpregnant women showed that both conjugate vaccines were safe and elicited specific antibody responses with opsonophagocytic killing of type V GBS [17]. Approximately 80 percent of vaccine recipients had a persistent antibody response for at least 2 years.
- A randomized, double-blind study with a GBS type V–TT vaccine tested in 32 healthy adults 65–85 years old demonstrated that the vaccine was safe and elicited specific antibody responses with opsonophagocytic killing of type V GBS and with 68 percent of recipients having a fourfold antibody increase [18]. The level of the specific antibody persisted up to 1 year, suggesting the potential for prevention of invasive type V GBS infections in healthy elderly people through vaccination.
- A Phase I, dose-escalating trial was conducted in 45 healthy adults to evaluate immunogenicity and reactogenicity of a GBS type V–TT vaccine ranging from 2.4 micrograms (mcg) to 38.5 mcg per dose [19]. The results showed that the vaccine was safe and elicited specific antibody responses with opsonophagocytic killing in all dose groups.
- Recently, a trial of a GBS vaccine in sexually active, nonpregnant women indicated that a vaccine to prevent GBS infection is possible [20]. This Phase II prospective, randomized controlled trial enrolled 668 healthy, sexually active nonpregnant women aged 18–45 years without GBS vaginal or rectal colonization at the time of their enrollment. The results showed that a GBS type III conjugate vaccine had an efficacy of 45 percent in preventing acquisition of vaginal type III colonization and an efficacy of 35 percent in preventing acquisition of rectal colonization over an 18-month period, when compared with participants who received the control tetanus and diphtheria toxoid vaccine.

In addition to the above studies, at least one pharmaceutical company has recently sponsored several studies to clinically evaluate a monovalent conjugate GBS vaccine [21].

Challenges and Future Opportunities

Although these efforts demonstrate progress in GBS vaccine development, several challenges remain:

1. Vaccine candidates that protect against multiple GBS subtypes must be developed. Serotypes Ia, Ib, II, III, and V are the predominant serotypes isolated from neonates, young infants, pregnant women, and adults with invasive GBS disease in the United States. Because antibodies against GBS CPS are serotype specific, a multivalent vaccine will be needed to provide broad protection. As a result, a number of formulation parameters, such as the number and amount of the protein carriers, will need to be optimized.
2. A correlate of immunity needs to be determined for the use of a GBS vaccine for maternal immunization. With the success of using antibiotics for prevention of neonatal sepsis, the number of cases of GBS neonatal sepsis in the United States has been reduced. Subsequently, it has been difficult to conduct the efficacy trials that are needed to reach this milestone.
3. There is a need for an established threshold for CPS type-specific antibody levels that correlate with protection. Although some data are currently available, information for all serotypes causing invasive GBS disease is required. Progress has been made in case-control comparisons of antibody levels to several GBS serotypes, including type III, in colonized mothers of infants with and without early-onset infection [22, 23]. This suggests that serotype-specific thresholds of protection can be set and will likely differ by serotype.
4. There is a need to standardize assays across laboratories for specific polysaccharide antibody levels and their biological functions.
5. Finally, additional industry commitment to GBS vaccine development is needed. Vaccine manufacturers' liability concerns have been an obstacle in the development of GBS conjugate vaccines to protect pregnant women from invasive GBS disease. The feasibility of maternal immunization has been demonstrated by the worldwide immunization of pregnant women for the prevention of neonatal tetanus, a major cause of infant mortality; however, safety data related to neonatal outcomes other than tetanus have not been collected. The risks involved in maternal immunization during the third trimester need to be better defined. The current use of inactivated influenza vaccine in pregnant women in the United States provides an opportunity to design studies to collect data to further demonstrate the safety and benefit of this approach to immunizing mother and infant.

Although NIAID's efforts in GBS vaccine development have focused on CPSs, an alternative strategy for prevention of GBS disease is to develop a vaccine based on a GBS surface protein. One advantage of this approach is that some of these proteins are immunogenic and do not need to be conjugated to other molecules. Also, recombinant DNA techniques can be used to produce large amounts of antigens for vaccine preparation.

Investigations with alpha and beta subunits of the GBS C protein, Rib protein, type V a-like and Rib proteins, and surface immunogenic protein (Sip) have demonstrated that these surface proteins are capable of eliciting antibody responses in mice and protecting against lethal bacterial challenges [24–27]. In addition to their use as immunogens, surface proteins have been used as carriers for CPS antigens. Compared with GBS CPS vaccines conjugated with TT, these conjugates have the advantage of enhancing the immunogenicity of the polysaccharide component of the vaccine and eliciting additional antibodies protective against GBS infections. Development of other formulations of GBS vaccines is another area of active research. A study with a bivalent vaccine composed of purified Rib and a proteins mixed with alum demonstrated an antibody response in mice and protected against lethal infection with GBS (serotypes Ia, Ib, II, and III) [28].

GBS C5a peptidase and beta-C protein are two surface proteins that have been conjugated to CPS antigens and are being pursued as vaccine candidates. Studies with anti-C5a peptidase antibodies demonstrated opsonic activity, suggesting that inclusion of C5a peptidase in a polysaccharide vaccine can produce another level of protection that is serotype independent [5].

A key development in the last decade includes a conserved pilus-based vaccine candidate that conferred protection against all tested GBS challenge strains in *in vitro* and *in vivo* studies [2]. In another study, a GBS CPS type III conjugated with recombinant cholera toxin B subunit administered intranasally improved the mucosal and systemic immune responses to GBS in a mouse model [29].

New strategies for GBS vaccine development include development of a universal GBS vaccine based on multiple genome screen technology. By analysis of the genome sequences of eight GBS isolates, more than 300 proteins were evaluated [30]. Four proteins that elicited protection in mice were selected, and their combination provided a high degree of protection against a large panel of strains that included all circulating serotypes.

Much progress has been made in the development of GBS vaccines during the last 30 years. Better CPS-conjugate vaccines have emerged, and the use of GBS proteins as immunogens or their conjugation to CPS offers a promising future for GBS vaccine development. However, these candidate vaccine components have yet to be studied in humans. Additional research is needed to expand serological findings to define protective levels of GBS antibodies and define immune defects in adults that result in invasive disease. There is also a need to better understand innate and adaptive responses of the immune system in relation to GBS pathogenesis in different populations. NIAID continues to fund basic research on GBS and supports both preclinical and clinical resources that may be helpful to academic and industry partners interested in collaborating on GBS vaccine development.

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REFERENCES

1. Sendi P, Johansson L, Norrby-Teglund A. Invasive group B streptococcal disease in non-pregnant adults. Infection. 2008;36:100-10.
2. Margarit I, Rinaudo CD, Galeotti CL, Maione D, Ghezzo C, Buttazzoni E, et al. Preventing bacterial infections with pilus-based vaccines: the group B streptococcus paradigm. J Infect Dis. 2009 Jan 1;199(1):108-15.
3. Verani JR, McGee L, Schrag SJ (Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC)). Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010. MMWR Recomm Rep. 2010 Nov 19;59(RR-10):1-36.
4. Jordan HT, Farley MM, Craig A, Mohle-Boetani J, Harrison LH, Petit S, et al. Revisiting the need for vaccine prevention of late-onset neonatal group B streptococcal disease: a multistate, population-based analysis. Pediatr Infect Dis J. 2008 Dec;27(12):1057-64.
5. Cheng Q, Carlson B, Pillai S, Eby R, Edwards L, Olmsted SB, et al. Antibody against surface-bound C5a peptidase is opsonic and initiates macrophage killing of group B streptococci. Infect Immun. 2001;69(4):2302-8.
6. Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999–2005. JAMA. 2008 May 7;299(17):2056-65.
7. Borchardt SM, DeBusscher JH, Tallman PA, Manning SD, Marrs CF, Kurzynski TA, et al. Frequency of antimicrobial resistance among invasive and colonizing group B streptococcal isolates. BMC Infect Dis. 2006 Mar 20;6:57.
8. Castor ML, Whitney CG, Como-Sabetti K, Facklam RR, Ferrieri P, Bartkus JM, et al. Antibiotic resistance patterns in invasive group B streptococcal isolates. Infect Dis Obstet Gynecol. 2008;2008:727505. Epub 2009 Feb 5.
9. Farley MM. Group B streptococcal disease in nonpregnant adults. Clin Infect Dis. 2001;33(4):556-61.
10. Brigtsen AK, Kasper DL, Baker CJ, Jennings HJ, Guttormsen HK. Induction of cross-reactive antibodies by immunization of healthy adults with types Ia and Ib group B streptococcal polysaccharide-tetanus toxoid conjugate vaccines. J Infect Dis. 2002 May 1;185(9):1277-84.
11. Baker CJ, Paoletti LC, Rench MA, Guttormsen HK, Carey VJ, Hickman ME, et al. Use of capsular polysaccharide-tetanus toxoid conjugate vaccine for type II group B streptococcus in healthy women. J Infect Dis. 2000 Oct;182(4):1129-38.
12. Baker CJ, Paoletti LC, Wessels MR, Guttormsen HK, Rench MA, Hickman ME, et al. Safety and immunogenicity of capsular polysaccharide-tetanus toxoid conjugate vaccines for group B streptococcal types Ia and Ib. J Infect Dis. 1999 Jan;179(1):142-50.
13. Baker CJ, Rench MA, McInnes P. Safety and immunogenicity of group B streptococcal type III capsular polysaccharide-tetanus toxoid conjugate vaccine in pregnant women. Clin Infect Dis. 2001;33:1151.
14. Baker CJ, Rench MA, Fernandez M, Paoletti LC, Kasper DL, Edwards MS. Safety and immunogenicity of a bivalent group B streptococcal conjugate vaccine for serotypes II and III. J Infect Dis. 2003 Jul 1;188(1):66-73.
15. Paoletti LC, Rench MA, Kasper DL, Molrine D, Ambrosino D, Baker CJ. Effects of alum adjuvant or a booster dose on immunogenicity during clinical trials of group B streptococcal type III conjugate vaccines. Infect Immun. 2001 Nov;69(11):6696-701.
16. Baker CJ, Rench MA, McInnes P. Immunization of pregnant women with group B streptococcal type III capsular polysaccharide-tetanus toxoid conjugate vaccine. Vaccine. 2003 Jul 28;21(24):3468-72.
17. Baker CJ, Paoletti LC, Rench MA, Guttormsen HK, Edwards MS, Kasper DL. Immune response of healthy women to 2 different group B streptococcal type V capsular polysaccharide-protein conjugate vaccines. J Infect Dis. 2004 Mar 15;189(6):1103-12.
18. Palazzi DL, Rench MA, Edwards MS, Baker CJ. Use of type V group B streptococcal conjugate vaccine in adults 65–85 years old. J Infect Dis. 2004 Aug 1;190(3):558-64.
19. Baker CJ, Rench MA, Paoletti LC, Edwards MS. Dose-response to type V group B streptococcal polysaccharide-tetanus toxoid conjugate vaccine in healthy adults. Vaccine. 2007 Jan 2;25(1):55-63.
20. Hillier SL, Ferris D, Fine P, Ferrieri P, Edwards M, Carey V, et al. Women receiving group B streptococcus serotype III tetanus toxoid (GBS III–TT) vaccine have reduced vaginal and rectal acquisition of GBS type III. Paper presented at: 47th Annual Meeting of the Infectious Diseases Society of America; 2009 Oct 29-Nov 1; Philadelphia, PA.
21. Novartis pipeline of new vaccines [Internet]. Cambridge, MA: Novartis vaccines. Available from: www.novartisvaccines.com/flash/products-diseases/pipeline.swf
22. Lin FY, Weisman LE, Azimi PH, Philips JB 3rd, Clark P, Regan J, et al. Level of maternal IgG anti-group B streptococcus type III antibody correlated with protection of neonates against early-onset disease caused by this pathogen. J Infect Dis. 2004 Sep 1;190(5):928-34. Epub 2004 Jul 28.
23. Lin FY, Philips JB 3rd, Azimi PH, Weisman LE, Clark P, Rhoads GG, et al. Level of maternal antibody required to protect neonates against early-onset disease caused by group B streptococcus type Ia: a multicenter, seroepidemiology study. J Infect Dis. 2001 Oct 15;184(8):1022-8. Epub 2001 Aug 31.
24. Areschoug T, Stalhammar-Carlemalm M, Larsson C, Lindahl G. Group B streptococcal surface proteins as targets for protective antibodies: identification of two novel proteins in strains of serotype V. Infect Immun. 1999;67(12):6350-7.
25. Brodeur BR, Boyer M, Charlebois I, Hamel J, Couture F, Rioux CR, et al. Identification of group B streptococcal Sip protein, which elicits crossprotective immunity. Infect Immun. 2000;68(10):5610-8.
26. Gravekamp C, Kasper DL, Paoletti LC, Madoff LC. Alpha C protein as a carrier for type III capsular polysaccharide and as a protective protein in group B streptococcal vaccines. Infect Immun. 1999;67:2491-6.
27. Lindahl G, Stålhammar-Carlemalm M, Areschoug T. Surface proteins of Streptococcus agalactiae and related proteins in other bacterial pathogens. Clin Microbiol Rev. 2005 Jan;18(1):102-27.
28. Larsson C, Stalhammar-Carlemalm M, Lindahl G. Protection against experimental infection with group B streptococcus by immunization with a bivalent protein vaccine. Vaccine. 1999;17:454-8.
29. Shen X, Lagergard T, Yang Y, Lindblad M, Fredriksson M, Holmgren J. Preparation and preclinical evaluation of experimental group B streptococcus type III polysaccharide-cholera toxin B subunit conjugate vaccine for intranasal immunization. Vaccine. 2000;19(7-8):850-61.
30. Maione D, Margarit I, Rinaudo CD, Masignani V, Mora M, Scarselli M, et al. Identification of a universal group B streptococcus vaccine by multiple genome screen. Science. 2005 Jul 1;309(5731):148-50.

CMV VACCINE SHOWS PROMISE

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Each year, approximately 8,000 infants in the United States develop severe hearing, mental, or movement impairments after becoming infected with cytomegalovirus (CMV), a common virus passed on to them while they are still in the womb. CMV is also the most common viral infection in patients who receive solid organ transplants, with up to 60 percent of transplant recipients developing symptomatic disease. Now, clinical trials supported by the National Institute of Allergy and Infectious Diseases (NIAID) have given rise to optimism that a vaccine to prevent CMV infection may be closer.

The first trial, led by pediatrician Robert Pass, M.D., of the University of Alabama at Birmingham, evaluated an experimental vaccine made from a single CMV protein, glycoprotein B, which is known to prompt an immune response. The candidate vaccine, known as CMV gB and supplied by Sanofi Pasteur, included an experimental adjuvant, MF59.

A total of 441 CMV-negative women, assigned at random to receive the candidate vaccine or a saline injection, were evaluated. Vaccinations were given to women within 1 year after they had given birth. Most women received three doses of trial vaccine or saline injection; all received at least one dose. In the final analysis, women who received the trial vaccine were 50 percent less likely to later become infected with CMV throughout the 42-month follow-up period than were women who received a saline injection.

In a second trial, led by Paul Griffiths, M.D., of the University College London Centre for Virology, the Sanofi Pasteur CMV gB vaccine was evaluated in volunteers awaiting liver or kidney transplants. A total of 67 patients received the vaccine, and 73 received a look-alike placebo. The vaccine was shown to be safe and immunogenic in all the volunteers who received it. Vaccination also reduced the posttransplant duration of viremia and the number of days of required treatment with the antiviral drug ganciclovir in patients who were seronegative at transplant but who received organs from donors who were CMV-positive.

An additional NIAID-supported Phase II trial of the experimental CMV vaccine is under way to evaluate the vaccine in healthy adolescent girls.

HIV/AIDS

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Overview

The impact of the HIV/AIDS pandemic has been profound and continues to have devastating effects worldwide. Although resources for HIV prevention and treatment have become increasingly available, the number of new infections remains at unacceptably high levels. In the United States, specific segments of the population—African Americans, Latinos, gay and bisexual men—are particularly vulnerable. And globally, in addition to the effects of the disease itself, affected populations are at higher risk for poverty, hunger, and childhood mortality. If current infection rates continue, it has been estimated that, as the need for expensive and ongoing treatment keeps pace, HIV-related costs could escalate to as high as $35 billion by 2030. The human and economic costs of HIV necessitate a preventive HIV vaccine.

The development of an HIV vaccine is complex and presents daunting scientific challenges due to HIV's unique characteristics, which include the ability to integrate into the genome of human cells without killing them and to destroy the immune system while evading the body's efforts to eliminate the virus. There are also many different genetic subtypes of HIV that circulate worldwide, and for a vaccine to be effective, it will need to induce immune responses that are broadly reactive to all or most of them.

The most rational way to design an effective vaccine is to identify the immune responses that protect against the specific infection and construct a vaccine that stimulates those responses. Because HIV can be transmitted through systemic and mucosal routes of exposure, by cell-associated and cell-free virus, researchers are working to identify the components of the immune system that are essential to inducing immunity and/or preventing or controlling infection. The two main types of immune responses are humoral immunity, which uses antibodies to defend against the virus, and cell-mediated immunity, which uses cytotoxic T lymphocytes (CTLs) to directly kill or control infected cells. The earliest vaccine research focused primarily on vaccines that elicited antibodies. Vaccine concepts involving a prime-boost combination strategy also have been tested. These vaccines stimulate a cellular immune response via CTLs (prime), as well as antibodies that bind to the virus (boost).

When a vaccine is developed, the hope is that it will be 100 percent effective in preventing infection. However, the first HIV vaccine may not be able to protect everyone from infection; it may be partially effective in preventing infection or only delay or prevent disease. Nonetheless, researchers recognize that such a vaccine could have a significant impact on the spread of new infections globally. With a decrease in the number of people susceptible to HIV infection, fewer people would be passing it on to others. If this occurs among a high percentage of people within a given population, new infections could be reduced dramatically or even eliminated. However, the benefits of a partially effective vaccine could be offset by relaxed practices of safe behaviors, education, and prevention resulting from perceived protection. Clearly, partially effective vaccines would need to be delivered in the context of a comprehensive prevention program. Thus, the National Institute of

HIV/AIDS Epidemic—Estimated Impact

Worldwide

- People living with HIV: 33.3 million
- People newly infected: 2.6 million
- Number of AIDS-related deaths: 1.8 million

United States

- People living with HIV: 1 million
- People newly infected: 56,300
- Percentage of people who don't know their HIV status: 21 percent

Sources: Joint United Nations Programme on HIV/AIDS (UNAIDS). Global report: UNAIDS report on the global AIDS epidemic 2010 [Internet]. Geneva (Switzerland): UNAIDS; 2010. Available from: www.unaids.org/globalreport/Global_report.htm

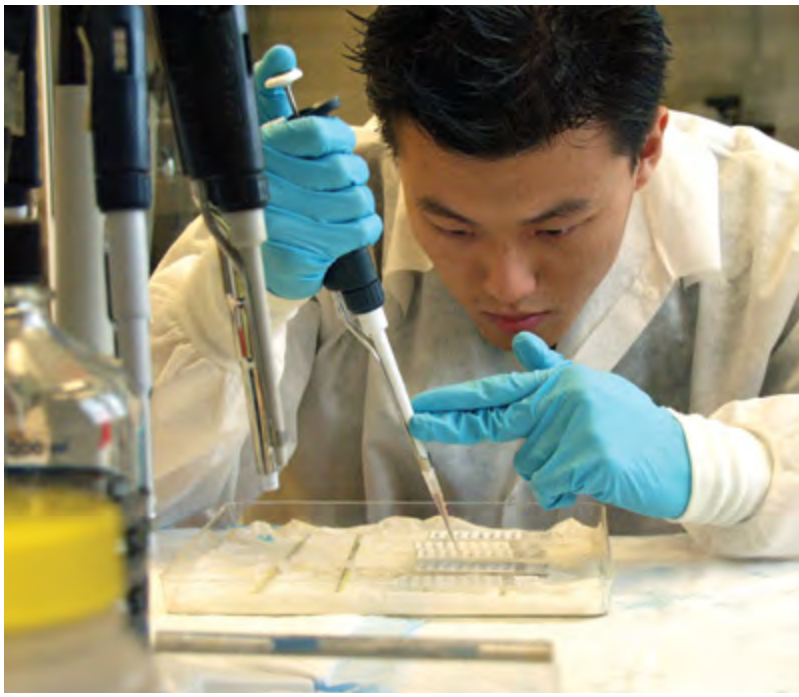
Centers for Disease Control and Prevention (CDC), Divisions of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention. HIV in the United States [fact sheet on the Internet]. Atlanta (GA): CDC; 2010 Jul. Available from: www.cdc.gov/hiv/resources/factsheets/us.htm

Allergy and Infectious Diseases' (NIAID's) HIV prevention research encompasses a variety of methods, such as topical microbicides, antiretroviral therapy (ART) to reduce the ability of HIV-infected persons to infect others, and pre-exposure prophylaxis (PrEP) to reduce the risk of HIV infection.

The Need for Partnership

Not only will multiple strategies be needed to fully prevent HIV, but also multiple organizational partners will be critical to identifying those strategies as quickly as possible. NIAID supports and oversees the vast majority of HIV vaccine research through collaborative partnerships with other government agencies, academic institutions, industry, private organizations and foundations, and the community. These partnerships greatly extend NIAID's scientific capacity, leverage resources (financial and otherwise), and encourage a coordinated approach that will potentially accelerate the development of an HIV vaccine. Among NIAID's partners and collaborators are the following:

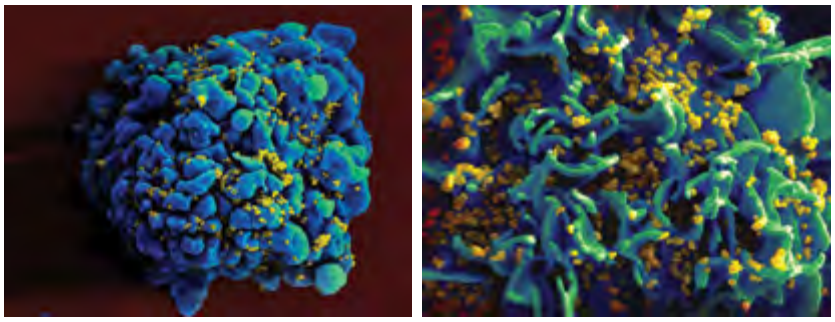
- **Global HIV Vaccine Enterprise (GHAVE).** GHAVE, also known as "the Enterprise," is a consortium of independent organizations, including NIAID, committed to accelerating the development of a preventive HIV vaccine. With its 2010 Scientific Strategic Plan, the Enterprise seeks to speed the development, execution, and analysis of HIV vaccine trials; better integrate preclinical and clinical research; capitalize on progress from recent HIV vaccine and other non-HIV research; and bring in new researchers from outside the field of HIV, as well as new funders. (See www.hivvaccineenterprise.org/scientific-strategic-plan.)
- **HIV Vaccine Trials Network (HVTN).** Funded by a cooperative agreement from NIAID, the HVTN is a clinical trials network of international scientists and researchers. The HVTN's mission is to evaluate candidate preventive HIV vaccines in all phases of clinical research, from evaluating experimental vaccines for safety and the ability to stimulate immune responses to testing vaccine efficacy, while at the same time generating information that will guide the design of improved vaccine concepts. (See www.hvtn.org.)
- **International AIDS Vaccine Initiative (IAVI).** IAVI was founded in 1996 to speed the discovery of an HIV vaccine; its partners include private companies, academic institutions, and government agencies, including the National Institutes of Health (NIH). (See www.iavi.org.)



Scientist at work. Courtesy of the U.S. Military HIV Research Program

- **NIAID HIV Vaccine Research Education Initiative (NHVREI).** NHVREI was established in 2006 as NIAID's primary mechanism for educating and fostering partnerships with key influencers within community-based, nonscientific organizations. The purpose of these partnerships is to promote understanding of and garner support for HIV vaccine research, especially among the more vulnerable and hard-to-reach populations. NHVREI is implemented through a contract with the Academy for Educational Development and Getting Your Message Right public relations. (See www.bethegeneration.nih.gov.)

  When the NHVREI contract expires in fall 2011, the scope of the project will be expanded to encompass all biomedical preventive research, including microbicides and PrEP. This effort, known as the Biomedical Prevention of HIV Research Education Initiative, will disseminate information on NIAID's prevention and vaccine clinical research activities and cultivate ongoing dialogue and relationships with key opinion leaders and organizations that reach highly affected populations.
- **South African AIDS Vaccine Initiative (SAAVI).** SAAVI was formed in 1999 to coordinate the research on and development and testing of HIV vaccines in South Africa. SAAVI is based at the Medical Research Council of South Africa and works with key national and international partners to identify an affordable, effective, and locally relevant AIDS vaccine. NIAID works collaboratively with SAAVI in



Scanning electron micrograph of HIV particles infecting a human T cell. LEFT: Image of an HIV infected H9 T cell, colorized by Anita Mora at RML. Image taken by Beth Schmidt in the Research Technologies Branch, Courtesy of NIAID. RIGHT: Close up view of an HIV infected H9 T cell, colorized by Anita Mora at RML. Image taken by Beth Schmidt in the Research Technologies Branch. Courtesy of NIAID

conducting HIV vaccine trials in South Africa. (See www.saavi.org.za.)

- **Community Advisory Boards (CABs).** NIAID highly values and actively seeks community input in all aspects of the research process. A key partner is the Global Community Advisory Board (GCAB), a group of community representatives who work with the leadership of the HVTN and site-specific CABs. CAB members help develop research plans, set research priorities, and participate as full members of protocol teams. CAB members also relay community needs and concerns, provide input on planned and ongoing research, and help assess the feasibility of a given trial in their community. (See www.niaid.nih.gov/topics/HIVAIDS/Research/Pages/outreach.aspx.)
- **U.S. Military HIV Research Program (MHRP).** MHRP was established in 1985 by the U.S. Army Medical Research and Materiel Command to protect U.S. troops entering areas with a high prevalence of HIV. Bringing together scientists from the U.S. Army, Navy, and Air Force, MHRP is dedicated to HIV vaccine development, HIV prevention, disease surveillance, and HIV care and treatment. NIAID jointly plans and executes HIV vaccine research projects and clinical trials with MHRP through an interagency agreement, which helps ensure that U.S. government-funded HIV vaccine research is well coordinated, efficient, and comprehensive. (See www.hivresearch.org.)

The Role of Basic Vaccine Research

The identification of new, improved candidate vaccines is urgently needed. Basic research in the fields of HIV natural history, pathogenesis, immunology, virology, viral and host genetics, and animal model development can lead to novel discoveries and increase our understanding of the earliest events in HIV infection and early immune responses. Scientific advances that define how the human immune system attempts to protect itself against HIV continue to unfold; provide a better understanding of the earliest events in natural infection, particularly in those who show an immune capacity to resist the virus; and are beginning to shape new vaccine approaches.

NIAID conducts basic research through the Dale and Betty Bumpers Vaccine Research Center (VRC) and the NIAID-funded Center for HIV/AIDS Vaccine Immunology (CHAVI), as well as individual grantees at academic centers throughout the United States. The VRC conducts research that facilitates the development of effective vaccines for human disease, with a primary focus on the development of vaccines for HIV/AIDS. The VRC's activities include basic research on envelope structure and potential targets for broadly neutralizing antibodies, new methodologies for enumerating protective T lymphocytes, and fundamental studies on adjuvants and potential vaccine vectors.

CHAVI is a virtual center designed to support intensive and highly collaborative projects that address key immunological roadblocks to the discovery and development of a safe and effective HIV vaccine. Established in 2005, this center currently focuses on elucidating early viral and immunological events and host genetic factors associated with HIV transmission, establishment of productive infection, and (partial) containment of virus replication; determining correlates of the simian form of HIV, simian immunodeficiency virus (SIV), immune protection in primates; designing, developing, and testing novel immunogens and adjuvants that elicit persistent mucosal and/or systemic immune responses in humans and primates; and advancing HIV vaccine candidates into early phase clinical trials.

In March 2008, following disappointing results from an NIAID-funded HIV vaccine trial (see page 126), NIAID held a summit on HIV vaccine research and development. In garnering input on how best to reinvigorate and advance HIV vaccine research, a scientific consensus emerged: Enormous advances in fundamental research are needed to design a safe and effective HIV vaccine. As a result, NIAID expanded and strengthened its portfolio of basic vaccine discovery research.

Two of NIAID's recent basic vaccine research programs are the Basic HIV Vaccine Discovery Research Initiative and the B Cell Immunology Partnerships for HIV Vaccine Discovery.

The Basic HIV Vaccine Discovery Research Initiative funds a broad range of basic research in areas such as immunology, virology, cellular and structural biology, and host genetics. The B Cell Immunology Partnerships for HIV Vaccine Discovery fosters cross-fertilization between B cell immunologists and HIV vaccinologists, seeking to facilitate discovery of novel vaccine design and immunization strategies for eliciting protective anti-HIV antibodies. Both programs have the potential of leading to new discoveries, expanded knowledge, and novel concepts and approaches applicable to HIV vaccine design.

Other important NIAID-funded basic research initiatives include the Phased Innovation Awards in AIDS Vaccine Research, which supports early stage AIDS vaccine research, and the Highly Innovative Tactics to Interrupt Transmission of HIV (HIT–IT). HIT–IT funds risky but rational approaches that could potentially provide long-term protection from acquiring HIV infection and that are based on newly gained knowledge of HIV pathogenesis, biology of HIV transmission, and human genetics. HIT–IT was specifically designed to attract investigators from outside the HIV research field, as well as those applying for their first grant.

Recent Progress

In 2010, scientists in NIAID's VRC discovered two potent human antibodies that can stop more than 90 percent of known global HIV strains from infecting human cells in the laboratory. It is hoped that these antibodies can be used to design improved HIV vaccines or can be further developed to prevent or treat HIV infection. The antibodies, known as VRC01 and VRC02, are naturally occurring and were found using a novel molecular approach that honed in on the specific cells that make antibodies against HIV. Both VRC01 and VRC02 were found to neutralize more HIV strains with greater overall strength than previously known antibodies to the virus. The atomic-level structure of VRC01 when attached to HIV also was determined, helping define precisely where and how the antibody attaches to the virus. With this knowledge, scientists have begun to design components of a candidate vaccine that could teach the human immune system to make antibodies similar to VRC01 and that might prevent infection by the vast majority of HIV strains worldwide [1, 2].

Basic research has led to a more thorough understanding of the earliest stages of HIV infection, including the "eclipse" phase, when HIV infection is becoming established but the virus is not yet detectable in the blood. CHAVI scientists also have examined "transmitter/founder" viruses by sequencing the genomes of viral particles in the plasma of 12 individuals prior to the emergence of HIV-specific immune responses. In 80 percent of heterosexual cases, they found that HIV infection stemmed from a single founder virus (range 1–6). In contrast, injection drug users were infected with a median of three viruses (range 1–16). Direct analysis of those viruses actually responsible for clinical infection may lead to important clues as to whether these viruses possess common features that could be effective targets for vaccine-induced immune responses [3, 4].

In another CHAVI study, uterine epithelial cells were identified as possible targets of HIV infection and transmission. Previously, the mechanisms of HIV transmission in the female reproductive tract were poorly understood. However, the likely exposure of these tissues to HIV is relevant to development of intervention strategies and may create a "window of vulnerability" that has not yet been systematically explored [5]. CHAVI scientists also characterized the critical role of the T-cell immune response in early virus control. Through analysis of host-immune responses to HIV infection, they showed that the first CD8+ T cells, despite limited breadth and very rapid virus escape, suppressed HIV as the amount of HIV in the blood was declining from a peak level. This implies that vaccine-induced HIV-specific T cells could contribute to the control of acute viremia (amount of HIV in the blood) if they are present before or early in HIV infection [6].

Preclinical Research

Discovery Strategies

Preclinical and clinical studies build on basic research findings and shed light on new and improved vaccine approaches. In addition to the B cell partnership program, which crosses from basic into discovery research, NIAID supports other preclinical initiatives, such as the HIV Vaccine Research and Design (HIVRAD) Program and Integrated Preclinical/Clinical AIDS Vaccine Development (IPCAVD) Program. These initiatives fund multidisciplinary research, including animal model development, immunogen structure, mechanism of vaccine action and vector development, and advanced-stage vaccine product development for investigators transitioning vaccines into human clinical studies. The HIVRAD and IPCAVD programs also foster and support public-private partnerships of scientists from industry and/or academia, to help advance promising vaccine concepts.

Through multiple contracts, NIAID also provides substantial resources for all phases of preclinical development and evaluation of candidate HIV vaccines, including *in vitro*

laboratory studies and *in vivo* testing in nonhuman primates. The Reagent Resource Support Program for AIDS Vaccine Development produces or purchases reagents needed for use in AIDS vaccine research, while the HIV Database and Analysis Unit compiles and analyzes data in several areas relevant to AIDS vaccine research. The unit encompasses the HIV Genetic Sequence Database, the HIV Molecular Immunology Database, and the Nonhuman Primate Vaccine Trials Database. Another important resource is the Preclinical Master Contract, which provides a complete spectrum of support for investigator-initiated vaccine development.

Recent Progress

The value of a T-cell-based HIV vaccine was brought into question after unexpected results from the Step Study (a Phase IIb proof of concept). The study's findings were announced in September 2007, when the trial was halted prematurely. This clinical trial enrolled individuals at high risk for HIV infection and evaluated a vector-based vaccine using recombinant adenovirus serotype 5 (rAd5), which is related to the virus that causes some forms of the common cold. The vector-based vaccine did not prevent HIV or significantly reduce set-point viral loads, or levels of infection, among study participants. However, research funded through the IPCAVD program recently demonstrated that an improved T-cell-based vaccine regimen using two distinct adenoviruses (rAd26 and rAd5) was able to substantially increase the protective efficacy, compared with an Ad5-based regimen in nonhuman primates. This improved regimen reduced viral set point and decreased AIDS-related mortality. The vaccine only expressed a single SIV antigen (Gag), suggesting that the partial immune control was mediated by a vaccine-elicited T-cell response (Gag-specific cellular immune response) rather than an antibody-based effect, since the vaccine lacked the SIV envelope protein [7].

Another important study found that a new HIV vaccination strategy using a "mosaic" design could expand the breadth and depth of immune responses in rhesus monkeys. The mosaic vaccine was designed through computational methods that created small sets of highly variable artificial viral proteins. When combined, these proteins theoretically could provide nearly optimal coverage of the diverse forms of HIV circulating in the world. In one NIAID-funded study, mosaic vaccines were embedded in specialized vectors designed to elicit strong T-cell responses. In rhesus monkeys, this vaccine resulted in a fourfold improvement in the monkeys' immune response, compared with previously tested vaccines, demonstrating that mosaic vaccines may improve the immune response against genetically diverse HIV–1 viruses [8–10].

VRC researchers also have developed a new "scaffold" strategy, which would teach the immune system to recognize certain protein structures on the viral surface and produce antibodies that bind to those structures and neutralize HIV. The technique involves extracting an epitope (an antibody-recognizable portion of the surface of a viral envelope protein) and placing the surface fragment into a different scaffold protein, which is intended to scaffold-lock the epitope in the shape recognized by the immune system. In theory, when a fixed epitope is introduced into an animal model (or eventually, a person), the immune system would recognize the envelope epitope and make antibodies against it. To test this scaffolding technique, VRC scientists applied it to an epitope on the surface of HIV that changes shape and is recognized by an HIV-neutralizing antibody known as 2F5. The epitope adopts a helical or spiral shape when removed from the surface of HIV, but the 2F5 antibody-recognizable version of it has an irregular, kinked shape. The scientists placed copies of the kinked epitope into scaffolds that locked it in that kinked form. Then the researchers injected these scaffold-bound epitopes into guinea pigs. In response, the animals' immune systems made antibodies very similar to 2F5 that bound tightly to the epitope. This study demonstrates that the engineering of protein scaffolds is a potentially useful approach in vaccine design. VRC researchers are continuing to refine this technique and apply it to the design of HIV vaccines, as well as vaccines for other infectious diseases [11].

Role of Nonhuman Primate Research

HIV vaccine testing in animal models continues to be an important step in evaluating the potential of vaccines. Nonhuman primate studies provide critical information regarding safety and potential efficacy, and help scientists understand how the body responds to infection. The hope is that, by examining the earliest events after mucosal infection (0–4 days) and the effects of vaccine interventions on those events, we will be able to learn more about how to prevent virus expansion beyond local mucosal tissue. Observing differences in these early interactions between animals that are successfully protected by vaccination and those that are not, and among different vaccine modalities, will provide valuable information for rational HIV vaccine design.

Although not ideal, nonhuman primates represent the best available surrogate model for research on AIDS pathogenesis

and vaccine development. Because HIV does not infect monkeys naturally, researchers conduct experiments with the closely related SIV. Combining parts of the HIV envelope and the inner core of SIV, researchers also have engineered chimeric simian-human immunodeficiency viruses (SHIVs) that mimic HIV infection and cause AIDS-like illness in macaque monkeys. Pathogenic chimeric SHIVs allow researchers to study the immune responses to the envelope-based HIV vaccines and the ability of these responses to stop or control the virus in a live model.

In addition to the HIVRAD and IPCAVD programs, NIAID carries out AIDS vaccine-related studies in the nonhuman primate model through the Simian Vaccine Evaluation Units (SVEUs). The SVEUs provide nonhuman primates for immunization with candidate SIV or HIV vaccines selected by NIAID, conduct initial assessment of the resulting immune responses, challenge the animals with infectious virus, determine parameters of infection, and collect samples for evaluation of immune responses and protection. NIAID also supports three Non-human Primate Core Immunology and Virology Laboratories contracts to carry out immunological and virological assessment of animals under study.

Recent Progress

Using a novel strategy previously developed by NIAID-funded researchers to identify transmitted HIV genomes in acutely infected people, researchers have been able to determine the molecular features of SIV transmission in experimentally infected macaques. They demonstrated that repeated intrarectal exposure of rhesus macaques to low doses of SIV replicates many of the features of human HIV mucosal transmission, at both the biological and molecular levels. Because an HIV vaccine will need to stop HIV at or near the moment it is transmitted across a mucosal membrane or in the early period before infection, this gives researchers a more reliable model to use in testing new vaccines and other preventive modalities [12].

Other NIAID-funded SIV research has shown that challenging monkeys with a cytomegalovirus (CMV)-based SIV vaccine results in containment of virus. Typically, virus replication and dissemination occurs within days after infection, whereas vaccine-induced T cell activation and recruitment to sites of viral replication takes weeks. Researchers hypothesized that vaccines designed to maintain activated effector memory T cells might impair viral replication at its earliest stage. They developed an SIV gene-containing vector based on rhesus CMV (RhCMV), because natural RhCMV infection in monkeys induces lifelong effector memory T-cell responses. In fact, when this vaccine was used in monkeys, it stimulated robust and persistent T-cell responses against all five proteins (Gag, Rev, Tat, Nef, and Env) encoded by the SIV genes inserted into the vector. Furthermore, these responses were generated regardless of preexisting immunity to RhCMV. When a low-dose challenge with a pathogenic SIV was

HERPEVAC TRIAL FOR WOMEN CONCLUDES

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In September 2010, a large-scale genital herpes vaccine trial called the Herpevac Trial for Women drew to a close. Supported by GlaxoSmithKline (GSK) Biologicals and the National Institute of Allergy and Infectious Diseases (NIAID), the Phase III clinical trial enrolled more than 8,000 women aged 18 to 30 years at 50 sites across the United States and Canada. Ultimately, results showed that the experimental vaccine, while safe and generally well tolerated, did not prevent genital herpes.

Genital herpes is estimated to affect 1 in 4 women in the United States, causing painful lesions or sores in the genital area. The disease has no cure; the causative herpes simplex virus (HSV) stays in the body permanently, where it can reactivate and cause periodic outbreaks. Herpes can lead to an increased risk of contracting HIV/AIDS and also can cause other health complications. For example, a woman with herpes can pass the disease on to her newborn, putting the baby at risk of serious brain, skin, or eye problems.

In earlier studies, the experimental herpes vaccine was found to prevent genital herpes infection in more than 70 percent of the female study volunteers who had no history of prior herpes virus infection, but it had no clear effect in the men. These studies formed the basis for the Herpevac Trial.

Although initial analysis of the Herpevac Trial results showed that the primary endpoint, prevention of herpes disease, was not accomplished, the trial was successful in many respects. Over 8 years of research, significant enrollment numbers and successful participant follow-up resulted in a firm conclusion. Data continue to be evaluated at this time, but one outcome is already clear: the results from the Herpevac Trial for Women will be an invaluable source of information to guide future research toward a new, improved vaccine to prevent genital herpes.

repeated, the vaccinated rhesus macaques showed increased resistance to acquisition of progressive SIV [13].

Scientists also have used a new approach to demonstrate that long-lasting neutralizing antibodies can be delivered by gene transfer *in vivo* and can provide continuous protection against SIV challenge. With this approach, the genes for SIV-specific antibodies are packaged into an adeno-associated virus (AAV) and then delivered by intramuscular injection. After AAV enters cells, those genes are expressed and result in production of the neutralizing antibodies. Intramuscular injection of this vaccine resulted in sufficient antibody production to protect against SIV infection in some animals and could provide a long-term method of producing antibodies without relying on the adaptive immune system of the host [14].

NIAID HIV Vaccine Trials as of June 2011

121 Cumulative Trials Conducted
- 112 Phase I
- 6 Phase II
- 2 Phase IIb
- 1 Phase III

19 Ongoing Trials
- 16 Phase I
- 2 Phase II
- 1 Phase IIb

Clinical Research

Background and Vaccine Concepts

At present, NIAID-supported HIV vaccine clinical trials are conducted primarily through the HVTN, a global network of international scientists and researchers whose mission is to evaluate preventive vaccines against HIV/AIDS. The HVTN conducts all phases of clinical research and, with sites in the United States, Africa, Asia, South America, and the Caribbean, spans four continents. An operations center, statistical and data management center, and central laboratory complete the network.

To date (June 2011), NIAID has supported a total of 121 HIV vaccine trials involving 79 products, 19 adjuvants, and approximately 29,500 trial participants. These trials have involved a number of different strategies, including component or subunit vaccines (made with a structural piece of HIV, such as an envelope or a core protein), live vector vaccines (a live bacterium or virus that transports genes that make HIV proteins), peptide (small pieces of HIV proteins) or fusion protein vaccines (two proteins merged together), DNA vaccines (direct injection of HIV genes), and vaccine combinations, such as a prime-boost strategy.

Early in the AIDS epidemic, most of the initial HIV vaccine research focused on component or subunit vaccines directed against the HIV envelope proteins gp160 and gp120, as they represent the primary targets for neutralizing antibodies in HIV-infected individuals. The first HIV vaccine clinical trial of a gp160 subunit candidate vaccine opened in 1987 at the NIH Clinical Center. The vaccine was tested in healthy, uninfected volunteers at low risk for HIV infection and caused no serious adverse effects. In 1992, NIAID launched the first Phase II HIV vaccine clinical trial, testing a recombinant subunit gp120 vaccine in uninfected volunteers at high risk for infection due to injection drug use, multiple sex partners, or sexually transmitted infections. Although these early vaccine candidates, as well as many others designed against the HIV envelope proteins, stimulated production of antibodies, antibody levels decreased within a relatively short period of time and rarely elicited CTLs.

Early studies also demonstrated that protection against HIV may require cell-mediated immune response, which involves the activation of specific CD8+ T cells that target HIV-infected cells. To elicit CD8+ T-cell responses, scientists employ viral or bacterial vectors to mimic infection by safely delivering specific HIV genes and inducing production of HIV proteins within cells. Because vectors only carry a small part of HIV genetic material, they cannot cause HIV infection. Different types of viral vector vaccines have been evaluated or are being evaluated, including poxviruses (e.g., canarypox and modified vaccinia Ankara (MVA), which is a weakened vaccinia virus), alphavirus, and Ad5. The canarypox vaccine was the first candidate HIV vaccine shown to induce a CTL response against diverse HIV genetic subtypes.

Researchers also have been exploring other possible vaccines, including DNA vaccines (containing one or more HIV genes or potential adjuvants). Vaccination, usually intramuscularly, will cause cells to take up the DNA and produce HIV proteins by normal cellular mechanisms, stimulating cell-mediated immune responses. Early studies demonstrated that

the first DNA candidates were safe, but did not induce strong immune responses. Subsequently, new technologies, such as codon-optimization and higher doses, were shown to enhance the performance of DNA vaccines.

In 1992, researchers turned their attention to a combination, or prime-boost, approach to improve the immunogenicity of HIV vaccines. Since then, prime-boost approaches have used combinations of DNA vaccines, viral vector vaccines, and subunit or peptide vaccines. Studies have shown the combination vaccine approach to be safe and immunogenic in volunteers at low and high risk for HIV infection, and that this approach can stimulate cellular immunity and the production of HIV-neutralizing antibodies.

Recent Progress

In late 2007, the HIV vaccine research field had disappointing news. The vaccine used in HVTN 502, also known as the Step Study, failed to prevent HIV infection and did not affect the level of viral load in those participants who were vaccinated but still became infected. More disturbingly, study participants—especially a subset of men who were uncircumcised and had naturally occurring neutralizing antibodies to Ad5 (the virus used to make the vaccine vector that delivered the HIV vaccine) at the time of enrollment—appeared to be at increased risk for infection. This study was terminated early as a result [15]. A related study, known as Phambili (HVTN 503), was evaluating the same adenovirus-based vaccine, and was suspended, as well.

The Step Study was testing Merck's vaccine candidate, the MRK Ad5 HIV–1 gag/pol/nef trivalent vaccine, based on a weakened adenovirus that had been altered to be rendered unable to replicate and infect humans. The study, involving 3,000 volunteers at high risk for acquiring HIV in regions with a high prevalence of HIV clade B, was designed to determine whether the vaccine either reduced HIV acquisition or lowered the viral set point in those volunteers who became infected.

Hoping to gain insight into the lack of efficacy, the HVTN laboratory program began evaluating HIV immune responses of Step Study volunteers who became infected during the study. Extensive analysis suggested that the immune responses induced by the vaccine put some early pressure on the virus, but did not have a significant impact on virus levels. A long-term follow-up study of participants, HVTN 504, was immediately launched to help researchers better understand the results; it evaluated the rate and risk of HIV infection among Step Study participants in the United States. Although there was an overall increased

CHLAMYDIA VACCINE BEING TESTED IN NONHUMAN PRIMATES

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The World Health Organization (WHO) estimates that more than 140 million people, mostly women and children in developing countries, are infected with the bacterium *Chlamydia trachomatis*, making chlamydia the most common bacterial disease in the world.

In the United States, chlamydia is perceived primarily as a "silent" disease that, despite no apparent symptoms in more than half of the infected population, can damage reproductive organs and cause infertility. Chlamydia is the leading reported sexually transmitted infection in the United States; in 2009, the Centers for Disease Control and Prevention reported approximately 1.2 million cases.

But chlamydia has an entirely different meaning in more than 50 developing countries, where infection is associated with the disease trachoma, which can cause blindness. WHO estimates that trachoma has left approximately 6 million people blind in Africa, the Middle East, Central and Southeast Asia, and Latin America. Trachoma causes the eyelid to fold inward and rub on the eyeball, abrading the corneal surface and resulting in impaired vision and blindness. Trachoma has been identified as one of the world's most neglected infectious diseases.

WHO hopes to eliminate blinding trachoma by 2020 through its SAFE strategy—Surgery, Antibiotics, Facial cleanliness, and Environmental change. Scientists at the National Institute of Allergy and Infectious Diseases (NIAID) are doing their part to complement this public health strategy by developing a vaccine to prevent trachoma.

The NIAID vaccine in development is designed to prevent infection from all 15 varieties of *C. trachomatis.* Researchers are testing the vaccine in nonhuman primates, following successful tests in cell culture and mouse models.

The focus of the vaccine is a protein antigen known as PmpD, or polymorphic membrane protein D, which was identified by NIAID's Harlan Caldwell, Ph.D. PmpD helps the bacteria infect host cells and suppress host immunity. Researchers are trying to learn whether a PmpD-based vaccine can generate multifunctional neutralizing antibodies capable of interfering with *C. trachomatis* infection and blocking the immunosuppressive effect of PmpD. One of the greatest challenges to fighting chlamydial infection, which the PmpD vaccine might solve, is that people do not develop a sustained protective immune response to the infection.

RV144 tested the "prime-boost" combination of two vaccines: ALVAC® HIV vaccine (the prime) and AIDSVAX® B/E vaccine (the boost). Courtesy of the U.S. Military HIV Research Program

risk of HIV among uncircumcised men, the higher rate of HIV acquisition was seen primarily during the initial vaccination phase of the trial, during the vaccination phase or the year thereafter (first 18 months), and then waned over time [16]. In another study, human leukocyte antigen (HLA) allele expression, which is known to influence progression of HIV disease and/or viral load set point, was significantly linked to viral load, although the effect did not appear to be mediated through increased breadth or magnitude of vaccine-induced responses; broader Gag responses may be associated with increased control of viral replication in Step Study vaccinees [17].

Following the early termination of the Step Study, plans to implement several other studies involving Ad5-based vaccines were put on hold or modified. One such vaccine being developed by the VRC consists of a multiclade recombinant Ad5-based component administered to boost immune responses induced by the prime DNA vaccine. A trial of this VRC vaccine regimen, HVTN 505, began as a small focused study with the primary goal of determining if the vaccine decreases viral load in study participants who later become infected with HIV. However, the trial was expanded in August 2011 so that it could also determine if the vaccine regimen prevents HIV infection. The results of RV144, discussed below, and a series of studies in nonhuman primates that showed that the VRC vaccine regimen prevented SIV infection 50 percent of the time in two-thirds of the monkeys tested, supported the expansion of HVTN 505. The study will now enroll a total of 2,200 participants and will evaluate if the VRC vaccine regimen is at least 50 percent effective in preventing HIV acquisition during the 18 months following immunization. As a safety precaution, participants must be circumcised and without Ad5 antibodies at the time they are enrolling. Although rAd5 is not likely to advance to licensure, this trial will generate useful information on the impact of the induced immune response on the virus and perhaps correlates for HIV vaccine protection.

NIAID is also supporting a number of other studies involving alternative adenovirus vectors, including a study of the VRC rAd5 combined with NYVAC (poxvirus vector) vaccine (HVTN 078), alternative lower seroprevalence rAd vectors (e.g., HVTN/IAVI study of an Ad26/Ad35 vaccine), and the VRC Ad5 vaccine with extensive mucosal assessment (HVTN 076). Several Phase I trials also are underway with the VRC Ad5 vaccine in collaboration with the HVTN to evaluate how delivery, timing, combinations, and host genetics influence the breadth and location of T-cell responses (HVTN 082, HVTN 083, HVTN 084, and HVTN 085).

Almost 2 years after the disappointing results of the Step Study, the field was infused with new optimism. Announced in September 2009, the Thai HIV Vaccine Trial, also known as RV144, showed that a candidate vaccine (based on a canarypox vector and gp120 protein) was 31 percent effective at preventing HIV infection. While the effect was modest, it was statistically significant. This was the first time an HIV vaccine had demonstrated an ability to prevent infection in people, and the trial thereby reinvigorated the field and gave us all a glimpse of what was possible [18]. (See page 126.)

The RV144 trial also provided the first opportunity to investigate immune correlates of vaccine efficacy in humans. Initial studies indicated that the antibody and T-cell responses were similar to those previously observed in studies using this regimen. Several RV144 working groups, which were established in the fall of 2009 and comprise various HIV vaccine stakeholder organizations and experts in the field, are working toward identifying the potential immune correlates of protection. NIAID also has established an HIV Mucosal

Immunology Group (MIG) program, which will share protocols for mucosal sample collection and assays to characterize and standardize the measurement of mucosal immune response across the field.

Future Directions

Discovery and Nonhuman Primate Research

While additional analysis of RV144 is expected to yield new information to increase scientists' understanding of how a highly effective HIV vaccine might work, it also will generate new questions. NIAID is positioned to answer the key questions with the efforts already underway and several new programs. One such program is the Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, which will seek to identify an immunogen that induces durable, highly effective, broadly protective immune responses. The program will support a multidisciplinary team of researchers focused on a number of critical scientific questions that require a "big science" approach.

The Innovation for HIV Vaccine Discovery initiative, which is designed to address gaps in HIV vaccine discovery, will fund basic research on new target molecules or pathways needed for designing an effective HIV vaccine. The HIVRAD program will continue to be an important component of NIAID's discovery effort, supporting projects for research that have advanced beyond the exploratory stage and that further address hypotheses crucial to vaccine design.

If additional analysis and follow-up studies from RV144 identify correlates of immunity, researchers will be able to optimize candidates that are already in the pipeline. However, in the absence of known correlates of protection, researchers will continue to seek other candidate vaccines and stimulate potentially protective immune responses.

Research studies also are being planned to explore the use of other vectors with greater immunogenicity, better adjuvants, and the use of additional protein boosts. Some vectors that have provided interesting results will be further investigated; these include replicating vectors (CMV, in particular), as well as vectored antibodies, which insert broadly neutralizing antibodies into a vector. In addition, mosaic inserts, described earlier, have already been studied in animals and have shown some success in enhancing the breadth and depth of immune responses. CHAVI researchers are currently designing the first human trial of a mosaic HIV vaccine candidate.

In 2011–2012, NIAID will establish the Consortia for AIDS Vaccine in Nonhuman Primates, to better understand the viral and host events that occur at the earliest stages of mucosal infection and the ways these events can be blocked or modulated by immunization. In addition, this program could help increase our understanding of the viral and host factors responsible for the nonpathogenic nature of SIV infection in natural host species.

Clinical Research

In future Phase IIb efficacy trials, NIAID will consider using an adaptive trial design so that a clear signal of efficacy can be identified early on. This would allow for changes to the trial design before the trial's natural conclusion. Specific milestones and points of analysis would be defined prior to trial initiation, and changes in trial design, based on what is learned at given time points, would be prescribed in advance.

In the wake of RV144, additional studies are being planned that could help identify potential correlates of protection and ultimately improvements to this or subsequent vaccine regimens. Because data from RV144 indicated that protection against HIV was highest at 6 to 12 months after vaccination, two smaller studies are being planned (RV305 and RV306) that will add a secondary boost to try to extend and increase early immune responses. In addition, Phase IIb trials are being planned to determine whether the results of RV144 can be extended to other populations (e.g., higher risk individuals) and regions (e.g., with higher incidence, with different clades, and in which different routes of transmission are predominant). These Phase IIb trials will seek to improve on the initial design with additional boosts and a different pox virus and/or different adjuvants.

NIAID will continue to pursue these and other clinical trials in collaboration with its many partners, including funded researchers and research organizations, government agencies, foundations, industry, and the community. By combining scientific resources, we hope to build on exciting new advances, continue to deepen the understanding of HIV vaccine design, and accelerate the development of an effective and safe HIV vaccine that can be used worldwide.

REFERENCES

1. Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, et al. Rational design of envelope surface identifies broadly neutralizing human monoclonal antibodies to HIV–1. Science. 2010 Aug 13;329(5993):856-61.
2. Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, Finzi A, et al. Structural basis for broad and potent neutralization of HIV–1 by antibody VRC01. Science. 2010 Aug 13;329(5993):811-7.
3. Bar KJ, Li H, Chamberland A, Tremblay C, Routy JP, Grayson T, et al. Wide variation in the multiplicity of HIV–1 infection among injection drug users. J Virol. 2010 Apr 7;84(12):6241-7.
4. Li H, Bar KJ, Wang S, Decker JM, Chen Y, Sun C, et al. High multiplicity infection by HIV–1 in men who have sex with men. PLoS Pathog. 2010 May 13;6(5):e1000890.
5. Ochsenbauer C, Ghosh M, Fahey J, Shen Z, Patel M, Ding H, et al. Susceptibility of epithelial cells from the human female upper reproductive tract to infection by transmitted/founder HIV–1. Paper presented at: AIDS Vaccine 2010. Hosted by the Global HIV Vaccine Enterprise and the Center for AIDS Research at Emory University; 2010 Sep 28–Oct 1; Atlanta, GA.
6. Goonetilleke N, Liu MK, Salazar-Gonzalez JF, Ferrari G, Giorgi E, Ganusov VV, et al. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV–1 infection. J Exp Med. 2009 Jun 8;206(6):1253-72.
7. Liu J, O'Brien KL, Lynch DM, Simmons NL, La Porte A, Riggs AM, et al. Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys. Nature. 2009 Jan 1;457(1):87-91.
8. Kong WP, Wu L, Wallstrom TC, Fischer W, Yang ZY, Ko SY, et al. Expanded breadth of the T-cell response to mosaic human immunodeficiency virus type 1 envelope DNA vaccination. J Virol. 2009 Mar;83(5):2201-15. Epub 2008 Dec 24.
9. Barouch DH, O'Brien KL, Simmons NL, King SL, Abbink P, Maxfield LF, et al. Mosaic HIV–1 vaccines expand the breadth and depth of cellular immune responses in rhesus monkeys. Nat Med. 2010 Mar;16(3):319-23. Epub 2010 Feb 21.
10. Santra S, Liao HX, Zhang R, Muldoon M, Watson S, Fischer W, et al. Mosaic vaccines elicit CD8+ T lymphocyte responses that confer enhanced immune coverage of diverse HIV strains in monkeys. Nat Med. 2010 Mar;16(3):324-8. Epub 2010 Feb 21.
11. Ofek G, Guenaga FJ, Schief WR, Skinner J, Baker D, Wyatt R, et al. Elicitation of structure-specific antibodies by epitope scaffolds. Proc Natl Acad Sci U S A. 2010 Oct 19;107(42):17880-7.
12. Keele BF, Li H, Learn GH, Hraber P, Giorgi EE, Grayson T, et al. Low-dose rectal inoculation of rhesus macaques by SIVsmE660 or SIVmac251 recapitulates human mucosal infection by HIV–1. J Exp Med. 2009 May 11;206(5):1117-34.
13. Hansen SG, Vieville C, Whizin N, Coyne-Johnson L, Siess DC, Drummond DD, et al. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. Nat Med. 2009 Mar;15(3):293-9.
14. Johnson PR, Schnepp BC, Zhang J, Connell MJ, Greene SM, Yuste E, et al. Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection. Nat Med. 2009 Aug;15(8):901-7.
15. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet. 2008 Nov 29;372(9653):1881-93.
16. McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, Janes H, et al. HIV vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. Lancet. 2008 Nov 29;372(9653):1894-905.
17. Frahm N, Janes H, Friedrich D, Krambink A, Slichter C, Smith R, et al. Beneficial effects of protective HLA class I allele expression and breadth of epitope recognition after vaccination on HIV viral load post infection. Poster presented at: AIDS Vaccine 2010. Hosted by the Global HIV Vaccine Enterprise and the Center for AIDS Research at Emory University; 2010 Sep 28–Oct 1; Atlanta, GA.
18. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV infection in Thailand. N Engl J Med. 2009 Dec 3;361(23):2209-20.

PROMISING HIV VACCINE TRIAL RESULTS: RV144, THE THAI HIV VACCINE TRIAL

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The field of HIV vaccine research was greatly encouraged when promising results of a preventive HIV vaccine trial in Thailand were announced in September 2009. This was the first time an investigational vaccine was shown to prevent HIV infection among some vaccinated individuals, giving the world great hope that a safe and effective HIV vaccine will one day become a reality.

Known as RV144 or the Thai HIV Vaccine Trial, this Phase III trial tested a prime-boost combination of two vaccine candidates (ALVAC–HIV and AIDSVAX B/E), which were based on the strains of HIV that commonly circulate in Thailand. The 6-year study, which began in 2003, was designed to test the vaccine regimen's safety and ability to prevent HIV infection, as well as its ability to reduce the amount of HIV circulating in the blood (the viral load) of those who became infected during the time they were participating in the study.

RV144 demonstrated that the vaccine was safe and that individuals who received the vaccine regimen were 31 percent less likely to contract HIV than those who received a placebo injection. Despite these encouraging results in preventing HIV infection, the vaccine regimen did not have an impact on viral load in those who became infected.

Scientists continue to examine the trial data to understand how the vaccine prevented HIV infections and determine whether the vaccine can be improved. The data are providing scientists with valuable insights that will guide the design and testing of future HIV vaccines.

RV144 was sponsored by the U.S. Military HIV Research Program and conducted jointly by the Thai Ministry of Public Health and U.S. Army. Specifically, the U.S. Army Medical Component of the Armed Forces Research Institute of Medical Sciences assisted with the conduct of the trial in Thailand.

Laboratories at the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand. Courtesy of the U.S. Military HIV Research Program

Major funding and other support were provided by the National Institute of Allergy and Infectious Diseases (NIAID), a component of the National Institutes of Health. The two vaccine products used in the trial were provided by Sanofi Pasteur (ALVAC–HIV) and Global Solutions for Infectious Disease (AIDSVAX B/E).

More than 16,000 non-infected men and women were enrolled in the study. Because the study was designed as a community-based trial, the volunteers were not selected based on HIV risk factors; they were mostly between 18 and 30 years of age and included individuals at both high and low risk of HIV infection. Approximately 40 percent of study participants were women.

Since the completion of RV144, trial collaborators and other experts in the field have been investigating what made this specific vaccine regimen work in some study participants. Ongoing studies hope to determine the specific types of immune responses responsible for protecting individuals from HIV infection. Identifying these "correlates of protection" would provide a critical measurement against which other vaccine products and approaches could be evaluated and optimized before taking them into large efficacy trials.

The knowledge gained from RV144 stands to benefit HIV prevention research efforts worldwide. NIAID will continue to work with its partners to develop and test potentially improved HIV vaccines.

For more information about RV144, please see:

MHRP: U.S. Military HIV Research Program [Internet]. Rockville (MD): The Program; c2011. RV144 Trial: Thai Phase III HIV Vaccine Trial; [cited 2011 Apr 28]; [about 3 screens]. Available from: www.hivresearch.org/research.php?ServiceID=13

NIAID: National Institute of Allergy and Infectious Diseases [Internet]. Bethesda (MD): The Institute; [updated 2009 Sept 24]. Press release, HIV vaccine regimen demonstrates modest preventive effect in Thailand clinical study; 2009 Sept 24 [cited 2011 Apr 28]; [about 2 screens]. Available from: www.niaid.nih.gov/news/newsreleases/2009/Pages/ThaiVaxStudy.aspx

Influenza

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Introduction

Influenza remains among the leading causes of vaccine preventable morbidity and mortality worldwide, with annual epidemics occurring in all age groups. In the United States, pneumonia and influenza together are among the top 10 causes of mortality, and between 1976 and 2007, the number of reported deaths associated with seasonal influenza ranged from 3,349 to 48,614 [1]. The World Health Organization (WHO) uses available country-specific data to estimate that each year seasonal influenza epidemics cause 3 to 5 million cases of severe illness and 250,000 to 500,000 deaths globally [2].

Despite prior vaccination or infection, susceptibility to influenza infection persists. As the virus replicates, mutations arise in its two main surface proteins: the hemagglutinin (HA) and neuraminidase (NA). Over time, new "versions" of the viruses emerge because they have accumulated enough mutations to antigenically alter these proteins (referred to as "antigenic drift"), rendering the population susceptible to reinfection and prior year influenza vaccines ineffective. As a result, the virus strains that will be used to produce influenza vaccines must be reviewed annually to see how closely they match the evolving strains that are circulating around the world and whether needed vaccine strains are updated to match those expected to cause the next epidemic.

The type of antigenic variation that results in a pandemic ("antigenic shift") occurs when a new type A influenza virus is introduced into the human population and that virus is able to transmit efficiently from person to person. Wild aquatic birds, such as ducks and shore birds, are the natural hosts of influenza A viruses, and strains containing one of the 16 known types of HA and one of the 9 known NA types have been isolated from birds. Co-infection of animals or humans with different influenza viruses can result in an exchange of their genetic material known as "reassortment," creating new forms of the virus. Influenza viruses that infect animals can also directly infect humans. Antigenic shifts resulting in pandemics occurred in the 1950s and 1960s, when reassortment resulted in the introduction of genes from, respectively, influenza HA-type 2 (H2) and HA-type 3 (H3) avian influenza viruses into human influenza viruses, and in 2009, when an influenza virus containing a mixture of genes that tracked back to swine, birds, and human sources was circulating in swine and directly infected humans, causing the first influenza pandemic of the 21st century [3].

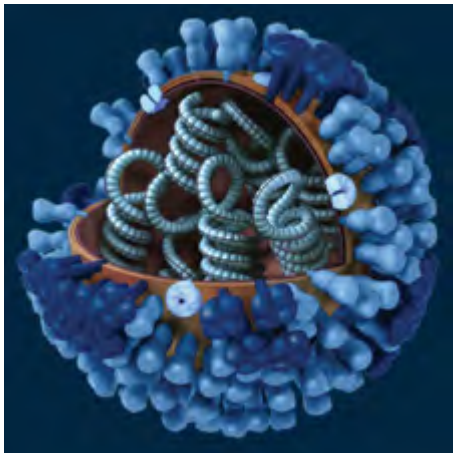
Flu Vaccines: First Steps to Today

Influenza vaccines are the primary means of preventing influenza disease and its related health complications. The first influenza vaccines were whole-virus vaccines produced by growing viruses in embryonated chicken eggs and inactivating them by chemical treatment. Clinical trials sponsored by the U.S. military conducted in the 1940s demonstrated that intramuscular administration of a dose of the inactivated virus was highly effective in preventing influenza illness in healthy young adults, provided there was a good match between the HA and NA proteins of the virus in the vaccine and those on the epidemic strain(s) [4]. Licenses were issued in 1945 to several companies in the United States for commercial production. Since the availability of eggs needed to manufacture influenza vaccines could be susceptible to an outbreak of avian influenza, there has been an investment by public and private sectors over the last decade to move to a cell culture-based manufacturing technology. Several companies have received regulatory approval in Europe using this approach, and in the United States, influenza vaccines produced in cell cultures are in late-stage clinical testing [5, 6].

In the United States, two types of influenza vaccines are Food and Drug Administration (FDA) approved to prevent seasonal influenza: trivalent inactivated vaccines (TIV) that are further purified into either split or subunit forms and administered via an intramuscular injection, and the live-attenuated influenza vaccine (LAIV), which is minimally purified and administered as a weakened form of the virus given as a nasal spray.

Over the last decade, several approved influenza vaccine manufacturers have left the U.S. market, and one of the National Institute of Allergy and Infectious Diseases' (NIAID's) efforts was to establish partnerships with the private sector to increase the availability of influenza vaccines. Through its clinical network of Vaccine and Treatment Evaluation Units (VTEUs),

NIAID has collaborated with the private sector to conduct clinical studies that helped support the approval of two new inactivated influenza vaccines in the United States [7, 8]. Additionally, much of the early stage research to support proof-of-concept studies on the intranasal LAIV (FluMist) was conducted by NIAID laboratories and in clinical trials supported by NIAID and the private sector. FluMist is currently approved to prevent influenza illness in healthy children and adolescents, aged 2 to 17 years, and healthy adults, aged 18 to 49 years.



3D graphical representation of a generic influenza virion's ultrastructure. A portion of the virion's outer protein coat has been cut away, which reveals the virus' contents. Courtesy of CDC

Research Aimed at Expanding Vaccine Options for Those at Greater Risk

For many years, the elderly were considered to be the population at greatest risk for health complications due to influenza, and pregnant women were identified to be at an increased risk during influenza pandemics. More recently, the substantial morbidity and mortality associated with influenza also has been recognized for very young children, individuals with underlying health conditions, and obese populations. As a result, in early 2010, the Centers for Disease Control and Prevention's (CDC's) Advisory Committee on Immunization Practices (ACIP) recommended annual influenza vaccination for all people 6 months of age and older for the upcoming influenza season unless the vaccine was contraindicated [9]. The ACIP noted that individuals may be unaware of whether they fall within a higher risk group and a "universal" recommendation sent a clear, more practical message [10].

A long-standing focus of the NIAID Influenza Program has been to better understand the breadth and duration of the immune response following influenza vaccination of "at-risk" populations and to identify strategies to improve vaccine effectiveness. Since early 2009, two studies with seasonal inactivated influenza vaccine and two studies with one or two doses of 2009 pandemic influenza vaccines have been initiated in pregnant women. To evaluate whether increasing the dosage of the vaccine also will increase the immune responses in the vulnerable age group of very young children, NIAID's VTEUs are currently conducting a study in which influenza vaccine-naive and fully primed 6- to 35-month-old children are being immunized with two doses of seasonal vaccine either at the currently recommended level (7.5 mcg of HA protein per strain) or at the standard adult dose (15 mcg of HA protein per strain). Results will compare data on safety and immunogenicity of the vaccines, and if the higher dosage results in higher antibody responses (which are thought to provide greater protection), they could support a recommendation that it be given routinely.

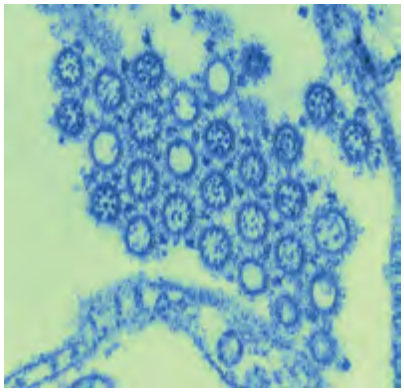
Collaboration With Industry on Developing a High-Dose Influenza Vaccine

Over the last 10 years, annual influenza vaccination rates in persons 65 years of age or older have steadily risen; however, the effectiveness of the current vaccine in preventing influenza illness in some elderly populations has been reported to be as low as 30 to 40 percent. NIAID-supported clinical investigators have conducted several studies to assess the safety and immunogenicity of high-dose vaccines in elderly and immunocompromised populations [11]. These data helped support an FDA approval of a high-dose influenza vaccine for individuals 65 years of age and older [12]. With the availability of this approved higher dose seasonal influenza vaccine, additional studies are being planned to look for possible benefits in other at-risk populations, including immunocompromised individuals.

Pandemic Influenza

The Vaccine Response to the 2009 H1N1 Pandemic

By May 2009, a few weeks after the 2009 H1N1 virus had first been reported, the virus was identified in more than 30 countries [13]. An urgent public health priority was the production of sample lots of vaccine that could be evaluated for safety and immunogenicity in U.S. government- and industry-supported clinical trials. Because the 2009 H1N1 virus contained a novel HA protein, the dosage of the vaccine and the number of doses needed to elicit a robust immune response was unknown. Under contract to the U.S. Department of Health and Human Services' (HHS') Biomedical Advanced Research and Development Authority (BARDA), approved manufacturers rapidly produced vaccine for their own and for NIAID clinical trials. Through its VTEUs, NIAID initiated three clinical trials in adults on August 7, 2009, to evaluate the safety of the inactivated 2009 H1N1 vaccine given alone or in combination



A highly-magnified, digitally-colorized transmission electron micrograph (TEM) depicting virions from an H1N1 influenza isolate. Courtesy of CDC

with the 2009–2010 inactivated seasonal influenza vaccine and the ability of these vaccines to induce protective levels of antibodies. Following a review of the safety data from the ongoing adult study, NIAID initiated similar studies in children aged 6 months to 17 years less than 2 weeks later. Within several weeks, preliminary results from NIAID's studies and independent studies conducted by vaccine manufacturers confirmed that a single 15 mcg dose of the vaccine elicited a robust immune response in healthy adults and older children. The NIAID studies also showed that while one dose of the vaccine generated significant antibody responses in pregnant women, children 9 years old and younger would need two doses of the vaccine [14]. These data were used to help inform vaccination recommendations for the 2009 H1N1 vaccines, which were approved by the FDA in September and distributed the first week of October. In collaboration with BARDA and influenza vaccine manufacturers, NIAID's VTEUs also completed a clinical study evaluating an inactivated 2009 H1N1 vaccine made by one company mixed with an oil-in-water emulsion adjuvant produced by a different company. In addition to assessing the safety and immunogenicity of combining these two products just prior to administration, the feasibility and logistics of this "mix-and-match" approach may serve as a guide for future pandemic preparedness and response efforts.

H5N1 Influenza Vaccines

In 1997, the highly pathogenic avian influenza (HPAI) H5N1 strain infected humans in Hong Kong directly from infected poultry. During this outbreak, 18 people became infected, 6 of whom died. The virus was successfully controlled with the culling of approximately 1.5 million chickens. In 2003, H5N1 viruses reappeared with two cases in family members from Hong Kong who had recently traveled to China.

Since 2003, H5N1 influenza viruses have caused outbreaks in 51 countries and have become endemic in avian populations in several countries (e.g., Indonesia and Egypt), resulting in 566 known human cases and 332 fatalities, primarily among poultry workers or others in close contact with domestic birds. Deaths occurred due to pneumonia, severe acute respiratory distress, or organ failure [15]. These ongoing outbreaks continue to raise concerns of an increase in human exposure to H5N1 viruses. Clustering of H5N1 cases suggests that limited human-to-human transmission has occurred among persons with intense, close contact; however, it is not yet known whether sustained human-to-human transmission of these viruses could be acquired through mutation alone or would require reassortment with currently circulating epidemic strains.

The public health community is concerned that H5N1 viruses may emerge as the next pandemic strain because of the number of human infections that have occurred. Recent pandemic preparedness efforts by NIAID have focused in large part on the clinical evaluation of influenza vaccines made using different forms of the H5N1 virus that have infected people in Asia. WHO reference laboratories have produced several reference virus strains for use in manufacturing vaccines against H5N1, using representative H5N1 strains, including A/HongKong/213/2003, A/Vietnam/1194/2004 (clade 1), A/Vietnam/1203/2004 (clade 1), A/Indonesia/5/2005 (clade 2.1), A/whooper swan/Mongolia/244/2005 (clade 2.2), A/bar-headed goose/Qinghai Lake/1A/2005 (clade 2.2), A/turkey/Turkey/1/2005 (clade 2.2.1), A/Anhui/1/2005 (clade 2.3.4), A/Egypt/1394-NAMRU4/2007-like (clade 2.2.1), A/goose/Guiyang/337/2006 (clade 4), and A/chicken/Vietnam/NCVD-016/2008 (clade 7).

In 1998, NIAID awarded a contract to Protein Sciences for the production of the first H5N1 vaccine, which was evaluated for safety and immunogenicity in a clinical trial conducted by the NIAID VTEUs [16]. In 2004, NIAID awarded contracts to Sanofi Pasteur and Chiron Corporation to support the production of vaccines against more recent forms of the virus for evaluation in adults, the elderly, and children. Over the last 6 years, NIAID has sponsored and/or supported, in collaboration with BARDA, more than 20 clinical trials to evaluate different dosage levels, routes of administration (intramuscular vs. intradermal), and studies with and without adjuvants. A series of studies also has been done showing that immunization with one H5N1 vaccine can prime for a more robust and broader cross-reactive antibody response following receipt with a second vaccine made from an antigenically distinct strain [17], as well as inactivated and live-attenuated H5N1 vaccines in a variety of populations, including healthy adults, the elderly, and children. One of the studies, a multicenter, double-blind

two-stage Phase I/II study using vaccine obtained under NIAID contract to Sanofi Pasteur, was conducted in healthy adults aged 18 to 64 years. The results from this trial were the basis of an FDA approval of the first H5N1 vaccine (two doses at 90 mcg vaccine for healthy adults) in 2007.

In 2005, NIAID announced a cooperative research and development agreement with MedImmune to produce and test LAIV for influenza A viruses with pandemic potential, beginning with vaccines for the highest priority HA subtypes, including H5. These vaccines are based on the same cold-adapted virus currently used for the licensed live-attenuated FluMist vaccine. However, like the inactivated vaccine used to manufacture vaccines for clinical trials, the HA gene of HPAI viruses will be modified to alter virulence determinants.

Both NIAID and MedImmune conduct laboratory studies to assess the safety of the vaccines before they are used for clinical trials. MedImmune is manufacturing the vaccines, and NIAID is testing the vaccines in an isolation unit. Clinical trials were initially conducted at Johns Hopkins Bloomberg School of Public Health's Center for Immunization Research in Baltimore, and are now being conducted at the University of Rochester in Rochester, NY, to assess vaccine safety, infectivity, and immunogenicity. Clinical trials of H5N1, H6N1, H7N3, and H9N2 vaccines have been completed. The vaccines were safe and well tolerated but were variably immunogenic.

New Vaccine Strategies

Over the last decade, a variety of new technologies have facilitated the development of innovative approaches to influenza vaccine development. NIAID and HHS through BARDA continue to encourage and supported multiple efforts to develop "next-generation" influenza vaccines.

Innovative vaccine strategies that do not require replication of the influenza virus are also being developed. This includes purified protein vaccines produced by recombinant DNA technology. These vaccines comprise individual viral proteins produced in cells and purified to a level not possible with vaccines started from a whole virus. These purified protein vaccines include formulations using only the HA protein, or the HA protein in combination with NA or internal proteins. Additionally, a variety of DNA vaccines are being developed. In these vaccines, viral DNA is included in a plasmid or viral vector, which, once injected in a person, enters the cells of the host, where it produces limited amounts of the viral proteins that elicit a specific immune response.

The ideal vaccine, one providing protection against any strain of influenza and not needing to be updated or administered every year to protect against newly emerging strains, is a goal not yet realized. However, research to develop such a universal vaccine is currently being supported by NIAID and others. One strategy being pursued is a "common epitope" vaccine, which utilizes highly conserved influenza proteins as targets. Although the HA and NA surface glycoproteins of influenza change frequently, many of the internal proteins are less variable. In particular, the M2 protein is being explored as a possible target. The M2 protein acts as an ion channel between the outside and inside of the virus membrane. A small portion of the M2 protein, its ectodomain or M2e, is exposed on the surface of the influenza virus. Although it is still in early stages of investigation, M2e may be an additional immune stimulus to augment the immune response and increase protection. A different type of common epitope vaccine focuses on the stalk region of the HA molecule, which is highly conserved, though immunorecessive. On removal of the immunodominant globular head region of HA, NIAID-supported investigators have generated a "headless HA" vaccine candidate that was shown to generate antisera with broader reactivity than those obtained from mice immunized with full-length HA. The headless HA provided full protection against death and partial protection against disease following lethal challenge in mice [18].

Innovative vaccine technologies provide new options to develop vaccines rapidly in response to a newly emergent strain. If successful, such advances could further increase vaccine production capacity and enhance preparedness against seasonal influenza and potential pandemic influenza strains [19].

REFERENCES

1. Centers for Disease Control and Prevention. Estimate of deaths associated with seasonal influenza—United States, 1976–2007. MMWR Morb Mortal Wkly Rep. 2010;59(33):1057-62.
2. Seasonal influenza [fact sheet N 211 on the Internet]. Geneva: World Health Organization; 2009. Accessed from: www.who.int/mediacentre/factsheets/fs211/en/index.html
3. Shinde V, Bridges CB, Uyeki TM, Shu B, Balish A, Xu X, et al. Triple-reassortant swine influenza A (H1) in humans in the United States. N Engl J Med. 2009;360:2616-25.
4. Grabenstein JD, Pittman PR, Greenwood JT, Engler RJ. Immunization to protect the US Armed Forces: heritage, current practice, and prospects. Epidemiol Rev. 2006;28:3-26. Epub 2006 Jun 8.
5. Frey S, Vesikari T, Szymczakiewicz-Multanowska A, Lattanzi M, Izu A, Groth N, et al. Clinical efficacy of cell culture-derived and egg-derived inactivated subunit influenza vaccines in healthy adults. Clin Infect Dis. 2010;51(9):997-1004.
6. Bernstein D. Cell culture-derived influenza vaccines: has their time come? Clin Infect Dis. 2010;51(9):1005-6.
7. Treanor J, Campbell JD, Brady RC, Keitel WA, Drame M, Jain VK, et al. Rapid licensure of a new, inactivated influenza vaccine in the United States. Hum Vaccin. 2005;1(6):239-44.
8. Talbot HK, Keitel W, Cate TR, Treanor J, Campbell J, Brady RC, et al. Immunogenicity, safety, and consistency of new trivalent inactivated influenza vaccine. Vaccine. 2008;26(32):4057-61.
9. CDC Online Newsroom. CDC's Advisory Committee on Immunization Practices (ACIP) recommends universal annual influenza vaccination [press release on the Internet]. Atlanta (GA): Centers for Disease Control and Prevention; 2010 Feb 24. Available from: www.cdc.gov/media/pressrel/2010/r100224.htm
10. Fiore A, Uyeki TM, Broder K, Finelli L, Euler G, Singleton JA, et al. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. MMWR Recomm Rep. 2010 Aug 6;59(RR-8):1-62.
11. Keitel WA, Atmar RL, Cate TR, Petersen NJ, Greenberg SB, Ruben F, et al. Safety of high doses of influenza vaccine and effect on antibody responses in elderly persons. Arch Intern Med. 2006;166:1121-7.
12. Falsey A, Treanor JJ, Tornieporth N, Capellan J, Gorse GJ. Randomized, double-blind controlled Phase 3 trial comparing the immunogenicity of high-dose and standard-dose influenza vaccine in adults 65 years of age and older. J Infect Dis. 2009;200(2):172-80.
13. World Health Organization. New influenza A (H1N1) virus infections: global surveillance summary, May 2009. Wkly Epidemiol Rec. 2009 May 15;84(20):173-9. Available from: www.who.int/wer/2009/wer8420/en/index.html
14. National Institute of Allergy and Infectious Diseases. 2009 H1N1 vaccine clinical trials [Internet]. Bethesda (MD): National Institute of Allergy and Infectious Diseases; 2010 Nov. Available from: www.niaid.nih.gov/topics/Flu/H1N1/ClinicalStudies/Pages/2009clinicalTrials.aspx
15. World Health Organization. Global Alert and Response (GAR)—Confirmed human cases of avian influenza A (H5N1) [Internet]. Geneva: World Health Organization; c2011. Available from: www.who.int/csr/disease/avian_influenza/country/en/
16. Treanor JJ, Wilkinson BE, Massoud F, Hu-Primmer J, Battaglia R, O'Brien D, et al. Safety and immunogenicity of a recombinant hemagglutinin vaccine for H5 influenza in humans. Vaccine. 2001 Feb 8;19(13-14):1732-7.
17. Belshe RB, Frey SE, Graham I, Mulligan MJ, Edupuganti S, Jackson LA, et al. Safety and immunogenicity of influenza A H5 subunit vaccines: effect of vaccine schedule and antigenic variant. J Infect Dis. 2011 Mar 1;203(5):666-73. Epub 2011 Jan 31.
18. Steel J, Lowen AC, Wang TT, Yondola M, Gao Q, Heye K, et al. Influenza virus vaccine based on the conserved epitope on the conserved hemagglutinin stalk domain. MBio. 2010 May 18;8(1). pii: e00018-10.
19. Gerhard W, Mozdzanowska K, Zharikova D. Prospects for universal influenza virus vaccine. Emerg Infect Dis. 2006;12(4):569-74.

NIAID CENTERS OF EXCELLENCE FOR INFLUENZA RESEARCH AND SURVEILLANCE

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The *National Institute of Allergy and Infectious Diseases* (NIAID) has a long history of supporting research activities to provide more effective approaches to controlling influenza virus infections. These activities include both basic and applied research on influenza basic biology and replication, pathogenesis, epidemiology, and clinical research to develop new and improved diagnostics, antiviral drugs, and vaccines. Due to the ever-present threat of an influenza pandemic, in 2007 NIAID established the Centers of Excellence for Influenza Research and Surveillance (CEIRS) to expand its worldwide influenza surveillance program and bolster influenza research in key areas, including understanding how the virus causes disease and how the immune system responds to infection with the virus. The goal of the CEIRS program is to provide essential information for the development of public health strategies crucial to both lessening the impact of seasonal influenza and responding to a pandemic.

Following the 2009 novel H1N1 influenza outbreak, the CEIRS sites quickly began work with the virus. The scientists used their infrastructure to provide essential information regarding the newly circulating virus. Some highlights of the CEIRS 2009 H1N1 research results include:

- First description of the origins and evolutionary genomics of the 2009 H1N1 virus [1].
- First description of the pathogenesis and transmission of the 2009 H1N1 virus in the ferret model [2].
- Detailed characterization of the 2009 H1N1 virus *in vitro* and *in vivo* and antiviral drug treatment after animal model infection with the virus [3].
- Description of the fitness of the 2009 H1N1 virus and the prediction that it would be the dominant influenza virus circulating for the upcoming influenza season [4].

From 2007 through 2011, CEIRS scientists published more than 450 peer-reviewed scientific journal articles and collected more than 475,000 influenza virus samples from multiple species—including wild birds, domestic poultry, swine, marine mammals, and humans. More than 17,000 influenza positive samples have been identified. In addition, more than 1,000 of these viral genomes have been fully sequenced and deposited in public databases. For more information, see www.niaid.nih.gov/research/resources/ceirs/.

Current activities of the CEIRS sites seek to expand the NIAID influenza virus surveillance program, both internationally and domestically, and to conduct research on such topics as the prevalence of avian influenza; how influenza viruses evolve, adapt, and are transmitted; and the immunological factors that determine whether an influenza virus causes only mild illness, severe illness, or death. Some sites will continually monitor international and domestic cases of animal and human influenza to rapidly detect and characterize viruses that may have pandemic potential and to generate pandemic vaccine candidates. The centers are laying the groundwork for new and improved control measures for emerging and re-emerging influenza viruses.

REFERENCES

1. Smith GJ, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature. 2009 Jun 25;459(7250):1122-5.
2. Munster VJ, de Wit E, van den Brand JM, Herfst S, Schrauwen EJ, Bestebroer TM, et al. Pathogenesis and transmission of swine-origin 2009 A (H1N1) influenza virus in ferrets. Science. 2009 Jul 24; 325(5939):481-3. Epub 2009 Jul 2.
3. Itoh Y, Shinya K, Kiso M, Watanabe T, Sakoda Y, Hatta M, et al. *In vitro* and *in vivo* characterization of new swine-origin H1N1 influenza viruses. Nature. 2009 Aug 20; 460(7258):1021-5.
4. Perez D, Sorrell E, Angel M, Ye J, Hickman D, Pena L, et al. Fitness of pandemic H1N1 and seasonal influenza A viruses during co-infection. PLoS Curr. 2009 Aug 25 [revised 2009 Aug 27]:RRN1011.

Malaria

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Malaria, caused by the parasite *Plasmodium falciparum* and related species, remains a major public health threat, especially among children and pregnant women in Africa. More than 500 million cases of malaria occur annually among the world's poorest populations [1], and this disease claims the lives of nearly 1 million children each year in Africa alone [2]. An effective malaria vaccine would be a valuable tool to reduce the disease burden and could contribute to eliminating malaria from some regions of the world. Current malaria vaccine candidates are directed against human and mosquito stages of the parasite's life cycle. RTS,S is the most advanced vaccine candidate because it has consistently demonstrated partial protection against malaria in Phase II clinical trials and in an ongoing Phase III trial in Africa [3]. New vaccine targets are being identified to improve the chances of developing a highly effective malaria vaccine.

The *P. falciparum* life cycle in humans is classified by three stages: the pre-erythrocytic stage (liver stage) that initiates the infection, the asexual erythrocytic stage (blood stage) that causes disease, and the gametocyte stage that infects the mosquitoes that transmit the parasite. Optimism that a safe and effective malaria vaccine can be developed is based on the fact that natural *P. falciparum* infection induces clinical immunity. In areas of intense *P. falciparum* transmission, where individuals are infected by hundreds of mosquito bites each year, immunity to severe, life-threatening disease is usually acquired early in childhood, whereas immunity to mild disease is not typically acquired until late adolescence. However, even in adults who have had decades of exposure to *P. falciparum*, sterile immunity to infection rarely develops and an occasional episode of fever can occur [4]. Thus, the immunity ultimately acquired by adults confers protection against the disease caused by the blood stages of *P. falciparum*, the stage in the life cycle of the parasite that causes symptoms in humans, and not protection from infection per se. The hope is that knowledge of the immune mechanisms and their *P. falciparum* targets that ultimately provide protection from disease in adults can be used to develop a vaccine that would induce in a child a facsimile of adult immunity. Alternatively, by understanding the clinically silent stages that precede the blood stage infection (i.e., sporozoite and hepatocyte stages), vaccination might be possible to evoke protective immune responses that do not normally develop in natural infection—namely, responses that prevent the blood stage infection from occurring at all. Both broad approaches to vaccine development are being taken [5, 6]. Compounding the difficulty of the vaccine effort are the large gaps in understanding *P. falciparum* infection biology, including how *P. falciparum* invades its target cells and causes disease.

Biologist checks culture volume in a fermenter growing *Pichia pastoris* yeast. This culture medium expresses a malaria antigen that the lab is evaluating for possible vaccine development. Courtesy of NIAID

Pre-Erythrocytic Stage Vaccines

The most advanced vaccine in development, RTS,S, consists of a recombinant protein expressed at the pre-erythrocytic stage that covers the parasite's surface—the circumsporozoite (CS) protein [7]. The idea of a pre-erythrocytic vaccine took shape with the seminal observation by Ruth Nussenzweig that vaccination of mice with irradiated sporozoites resulted in protection [8] and that protection could be achieved by immunization with the CS protein alone [9]. Development of pre-erythrocytic vaccines began with cloning of the *P. falciparum* CS protein [10] and collaboration in 1985 between the Walter Reed Army Institute of Research and industry partners. This research led to the development of the RTS,S vaccine. RTS,S consists of hepatitis B surface antigen (HBsAg) particles fused to the CS protein and formulated with the adjuvant AS01 [7, 11]. In a series of Phase II clinical trials, 30–50 percent of malaria-naive adults immunized with RTS,S were protected against challenge by mosquitoes that were infected with the

homologous *P. falciparum* clone [11–16]. For this vaccine, protection correlated with CS-specific antibody and CD4+ T-cell responses [16], but re-analysis of the data suggests that the contribution of T-cell immunity to protection may be minimal [17]. In Phase II field trials in The Gambia [18] and Kenya [19], RTS,S conferred short-lived protection against malaria infection in approximately 35 percent of adults, but the results from the trial in Kenya did not reach statistical significance. Among children and infants who were immunized with RTS,S in Phase II trials conducted in Mozambique, Tanzania, and Kenya, approximately 30–50 percent were protected from clinical malaria [20–24], but protection was generally short-lived. In field trials, immunization with RTS,S induced antibodies that correlated with protection from *P. falciparum* infection [25, 26] but not clinical disease [20, 24, 25, 27].

The RTS,S vaccine entered Phase III clinical trials in 2009. Based on results from Phase II trials, RTS,S is likely to provide only partial protection. However, precluding any unpredictable adverse effects, the vaccine could benefit millions of children by substantially reducing malaria morbidity and mortality. Initial results of the Phase III trial indicate that the RTS,S vaccine reduces episodes of clinical malaria by half in children aged 5–17 months over the first year of follow-up. Efficacy and safety results in 6- to 12-week-old infants, and longer term protectivie effects of the vaccine, are expected by the end of 2014 [3]. Efforts to improve the efficacy of CS protein–based vaccines with alternative adjuvants [28] or viral vectors [29, 30] have been unsuccessful to date, but several studies are still ongoing. Preclinical research efforts are focusing on inducing higher levels of CS protein–specific antibody [31]. In one study, the CS repeat peptide conjugated to the mosquito stage ookinete surface protein Pfs25 induced high levels of uncommonly long-lasting antibodies to both vaccine components in mice [31]. In principle, this vaccine strategy could confer protection against liver infection and block transmission by the mosquito vector.

THE INTERNATIONAL CENTERS OF EXCELLENCE FOR MALARIA RESEARCH

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A major resurgence of interest in and funding for malaria research, control efforts, and new product development has occurred during the last decade. Several successes have emerged from these investments, ranging from sequencing of the genomes of *Plasmodium falciparum, Plasmodium vivax,* and *Anopheles gambiae*, to more applied areas such as improved drugs, diagnostics, and insecticides, as well as to public health interventions such as widespread use of long-lasting insecticide-treated bed-nets and highly effective artemisinin combination therapies. According to the World Health Organization's *World Malaria Report 2010,* many malaria-endemic countries are presently experiencing a decrease in the incidence of malaria after years of increase or stagnation. Despite these recent gains, basic epidemiological information about the "malaria reality" on the ground in several endemic countries is still lacking.

In 2010, the National Institute of Allergy and Infectious Diseases, a component of the National Institutes of Health, established 10 International Centers of Excellence for Malaria Research (ICEMRs) to address some of the malaria research gaps that currently exist in global endemic settings, including parts of Africa, the Pacific Islands, and Latin America.

Renewed involvement and commitment by research institutions, control programs, governments, and funding agencies has resulted in a rapid scale-up of access to malaria control measures, which in turn are changing the landscape of malaria. With centers located in every malaria-endemic region of the world, the ICEMRs are uniquely positioned to capture this shifting epidemiology in real time across the globe, and these data will inform future malaria control and elimination programs.

Several features of the ICEMRs distinguish them from other initiatives. Most observational studies in malaria are restricted to a single field site with a relatively homogeneous population. In contrast, each ICEMR has multiple field areas, which are thought to be distinct with respect to disease transmission and burden. It is anticipated that data gathered from these heterogeneous sites, using a common study design, may provide an opportunity to generalize the findings beyond the study areas. All centers are adopting a multidisciplinary approach to study the complex interactions between the human host, the malaria parasite, the vector, and the ecology at the molecular, cellular, organismic, population, and field levels. It is expected that such studies will provide the knowledge base necessary for improved clinical and field management of malaria, as well as guide the development of new tools and interventions.

Efforts also are ongoing to develop vaccines that induce T-cell immunity to the pre-erythrocytic stage through either irradiated [32] or genetically attenuated [33] sporozoites, or through expression of *P. falciparum* liver stage proteins in viral vectors [34]. The irradiated sporozoite strategy is based on an observation that the bites of irradiated infected mosquitoes protected humans from challenge with infected mosquitoes that were not irradiated [35], suggesting that irradiated sporozoites in humans could be an effective vaccine—just as effective as they were first shown in mice [8]. This approach is not practical because protection required the bites of more than 1,000 infected, irradiated mosquitoes [36]. As an alternative, manufacturing processes have been developed to purify and cryopreserve irradiated sporozoites from aseptic mosquitoes in the quantities necessary for vaccination [32]. In the first clinical trial, the irradiated, purified, and cryopreserved sporozoite vaccine was safe and well-tolerated but only modestly immunogenic and protected only a few individuals. The next clinical trial will attempt to improve efficacy by optimizing the route of administration [37]. Studies are also in progress to determine whether sporozoites can be attenuated for use as vaccines by methods other than irradiation [33, 38]. A Phase II trial is underway to test this strategy in humans.

In mouse models of malaria, immunization with irradiated sporozoites induces CD8+ T cells that kill parasite-infected hepatocytes. The known targets of CD8+ T-cell killing, in addition to CS protein, include thrombospondin-related anonymous protein (TRAP) and liver stage antigen (LSA). In *P. falciparum*–naive adults, immunization with viral vectors containing TRAP peptides led to partial protection from challenge by infected mosquitoes through mechanisms that involved the induction of large numbers of TRAP-specific interferon gamma (IFNγ)-producing T cells [39]. Disappointingly, this vaccine did not induce protection in children in Africa [40]. For unknown reasons, the level of TRAP-specific INFγ-producing T cells was considerably lower in vaccinated African children compared with that in *P. falciparum*–naive adults [39, 40]. Efforts are ongoing to improve the T-cell immunogenicity of TRAP with simian adenovirus vectors [34].

Asexual Erythrocytic Stage Vaccines

The asexual blood stage of the parasite's life cycle begins with the release of merozoites into the bloodstream from ruptured infected hepatocytes. The blood stage is the only stage in the parasite's life cycle that causes disease [41]. Because immunity to disease develops with repeated *P. falciparum* infections, the acquisition of naturally acquired immunity by a vaccine may be able to be mimicked and accelerated. One key component of blood stage immunity is antibodies. This was demonstrated by experiments in which the transfer of immunoglobulin G from immune, adult Africans to partially immune African [42] or Thai [43] children rapidly reduced parasitemia and fever. These experiments suggest that a vaccine could theoretically be developed that would elicit in children the antibodies that protect against disease in adults. At present, the specificity of antibodies that confer protection against malaria is not fully characterized, and the precise mechanisms of antibody-mediated protection are unknown.

Several blood stage antigens are in clinical development as vaccines:

- Apical membrane antigen 1 (AMA1) [44]
- Erythrocyte binding antigen-175 (EBA–175) [45]
- Glutamate-rich protein (GLURP) [46, 47]
- Merozoite surface protein 1 (MSP1) [48]
- Merozoite surface protein 2 (MSP2) [49]
- Merozoite surface protein 3 (MSP3) [46, 50–52]
- Serine-rich antigen 5 (SERA5) [52]

All of these antigens are highly expressed on the surface of the merozoite. Unfortunately, recent Phase II trials of the most advanced blood-stage candidates, AMA1 and MSP1, did not demonstrate efficacy in African children [44, 48]. Efforts are ongoing to enhance the vaccine efficacy of AMA1 and MSP1 with novel adjuvants [54, 55] or viral-vectored prime-boost strategies [34] or by combining AMA1 and MSP1 [56]. However, extensive parasite genetic diversity, due to the selective pressure exerted by the human immune response, presents a major hurdle for the development of blood stage vaccines [57, 58]. For example, the AMA1 antigen is highly polymorphic, with hundreds of haplotypes that affect the ability of antibodies specific for one haplotype to block invasion by other haplotypes [59]. Unless strategies are developed to overcome such genetic diversity, highly polymorphic *P. falciparum* antigens, such as AMA1, are unlikely to be useful [57, 59].

Combining Pre-Erythrocytic and Erythrocytic Stage Vaccines

The World Health Organization's guidelines for measuring the efficacy of malaria vaccines in Phase III clinical trials recommend defining the primary endpoint to the time of the

first clinical malaria episode [60]. By these criteria, the RTS,S vaccine has demonstrated 30–50 percent efficacy in Phase II trials [27]. Preliminary data from an ongoing Phase III trial are consistent with these results [3]. However, an important unanswered question remains: How does partial pre-erythrocytic immunity influence the time to onset of clinical malaria, which occurs during the erythrocytic stage? One possibility is that a partially effective pre-erythrocytic vaccine reduces the number of infected hepatocytes, thus decreasing the number of merozoites that are released into the bloodstream and allowing more time for blood stage immunity to develop before the fever threshold is reached. If so, combining *P. falciparum* antigens that target the pre-erythrocytic and blood stages may further decrease the probability of reaching the disease threshold. This eventuality provides the rationale for several multistage vaccine candidates that are currently being evaluated in clinical trials.

Transmission-Blocking Vaccines

Transmission-blocking malaria vaccine candidates target antigens on gametes, zygotes, or ookinetes in the mosquito midgut. Antibodies induced in the human blood by these vaccine candidates and ingested with the parasite can block the parasite's life cycle development in the mosquito [61]. These vaccines could be important tools to eliminate malaria and protect against epidemics if *P. falciparum* parasites are reintroduced after a period of elimination. A transmission-blocking malaria vaccine would not confer protection to the vaccinated individual unless it is combined with an effective pre-erythrocytic [31] or erythrocytic vaccine.

P. falciparum proteins, such as Pfs25, that are expressed only in the mosquito are not polymorphic because they are not under adaptive immune pressure in the human host [62]. Gamete proteins, such as Pfs48/45 and Pfs230, which are expressed in the human host, are more polymorphic than Pfs25, but still have conserved domains that are present in all parasite clones studied to date [63]. Pfs230 has the additional advantage of being the target of antibody-dependent complement lysis [64]. In a mouse model, antibodies to HAP2, a *Plasmodium* protein thought to be involved in the fusion of male and female gametes in the mosquito midgut [65], also have transmission-blocking activity *in vivo* and *in vitro* [66].

Current evidence suggests that the levels of antibodies in blood that would be required to significantly affect parasite development in the mosquito may need to be extremely high [67]. Conjugation of Pfs25 to a carrier, such as outer membrane protein complex (OMPC) of *Neisseria meningitidis* serogroup B, may overcome this problem, because the conjugate induces high titer antibodies in rhesus monkeys that persist for at least 2 years [68]. Preclinical and clinical development of transmission-blocking vaccines is underway because of their promise for malaria elimination.

Conclusion

Malaria is a complex parasitic disease that imposes an enormous disease burden, and for which a vaccine is not currently available. Optimism that a vaccine can be developed comes from observations that malaria immunity can be acquired through natural and experimental infection. However, many *P. falciparum* proteins are highly polymorphic and their biological functions are redundant, resulting in significant challenges to vaccine design. Nevertheless, by recruiting experts in all aspects of *P. falciparum* infection biology and immunity to work on this problem, the development of a highly effective malaria vaccine may be possible.

REFERENCES

1. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature. 2005;434:214-7.
2. World Health Organization (WHO). World Malaria Report 2010. Geneva: WHO Press; 2010. 238 p.
3. The RTS,S Clinical Trials Partnership. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. N Engl J Med [Internet]. 2011 Oct 18. [Epub ahead of print]. Available from: www.nejm.org/doi/full/10.1056/NEJMoa1102287.
4. Marsh K, Kinyanjui S. Immune effector mechanisms in malaria. Parasite Immunol. 2006;28:51-60.
5. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, et al. Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature. 2002;419:498-511.
6. Florens L, Washburn MP, Raine JD, Anthony RM, Grainger M, Haynes JD, et al. A proteomic view of the *Plasmodium falciparum* life cycle. Nature. 2002;419:520-6.
7. Casares S, Brumeanu TD, Richie TL. The RTS,S malaria vaccine. Vaccine. 2010;28:4880-94.
8. Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of *Plasmodium berghei*. Nature. 1967;216:160-2.
9. Nussenzweig V, Nussenzweig RS. Development of a sporozoite malaria vaccine. Am J Trop Med Hyg. 1986;35:678-88.

10. Dame JB, Williams JL, McCutchan TF, Weber JL, Wirtz RA, Hockmeyer WT, et al. Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite *Plasmodium falciparum*. Science. 1984;225:593-9.

11. Gordon DM, McGovern TW, Krzych U, Cohen JC, Schneider I, LaChance R, et al. Safety, immunogenicity, and efficacy of a recombinantly produced *Plasmodium falciparum* circumsporozoite protein-hepatitis B surface antigen subunit vaccine. J Infect Dis. 1995;171:1576-85.

12. Stoute JA, Slaoui M, Heppner DG, Momin P, Kester KE, Desmons P, et al.; RTS,S Malaria Vaccine Evaluation Group. A preliminary evaluation of a recombinant circumsporozoite protein vaccine against *Plasmodium falciparum* malaria. N Engl J Med. 1997;336:86-91.

13. Kester KE, McKinney DA, Tornieporth N, Ockenhouse CF, Heppner DG, Hall T, et al. Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. J Infect Dis. 2001;183:640-7.

14. Kester KE, McKinney DA, Tornieporth N, Ockenhouse CF, Heppner DG Jr, Hall T, et al. A Phase I/IIa safety, immunogenicity, and efficacy bridging randomized study of a two-dose regimen of liquid and lyophilized formulations of the candidate malaria vaccine RTS,S/AS02A in malaria-naive adults. Vaccine. 2007;25:5359-66.

15. Kester KE, Cummings JF, Ockenhouse CF, Nielsen R, Hall BT, Gordon DM, et al. Phase 2a trial of 0, 1, and 3 month and 0, 7, and 28 day immunization schedules of malaria vaccine RTS,S/AS02 in malaria-naive adults at the Walter Reed Army Institute of Research. Vaccine. 2008;26:2191-2202.

16. Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, et al. Randomized, double-blind, Phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. J Infect Dis. 2009;200:337-46.

17. Olotu AI, Fegan G, Bejon P. Further analysis of correlates of protection from a Phase 2a trial of the falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naive adults. J Infect Dis. 2009;201:970-1.

18. Bojang KA, Milligan PJ, Pinder M, Vigneron L, Allouecheé A, Kester KE, et al. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. Lancet. 2001;358:1927-34.

19. Polhemus ME, Remich SA, Ogutu BR, Waitumbi JN, Otieno L, Apollo S, et al. Evaluation of RTS,S/AS02A and RTS,S/AS01B in adults in a high malaria transmission area. PLoS One. 2009 Jul 31;4(7):e6465.

20. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Milman J, et al. Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomised controlled trial. Lancet. 2004;364:1411-20.

21. Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E, Aide P, et al. Duration of protection with RTS,S/AS02A malaria vaccine in prevention of *Plasmodium falciparum* disease in Mozambican children: single-blind extended follow-up of a randomised controlled trial. Lancet. 2005;366:2012-8.

22. Sacarlal J, Aide P, Aponte JJ, Renom M, Leach A, Mandomando I, et al. Long-term safety and efficacy of the RTS,S/AS02A malaria vaccine in Mozambican children. J Infect Dis. 2009;200:329-36.

23. Abdulla S, Oberholzer R, Juma O, Kubhoja S, Machera F, Membi C, et al. Safety and immunogenicity of RTS,S/AS02D malaria vaccine in infants. N Engl J Med. 2008;359:2533-44.

24. Bejon P, Lusingu J, Olotu A, Leach A, Lievens M, Vekemans J, et al. Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. N Engl J Med. 2008;359:2521-32.

25. Guinovart C, Aponte JJ, Sacarlal J, Aide P, Leach A, Bassat Q, et al. Insights into long-lasting protection induced by RTS,S/AS02A malaria vaccine: further results from a Phase IIb trial in Mozambican children. PLoS One. 2009;4(4):e5165.

26. Aponte JJ, Aide P, Renom M, Mandomando I, Bassat Q, Sacarlal J, et al. Safety of the RTS,S/AS02D candidate malaria vaccine in infants living in a highly endemic area of Mozambique: a double blind randomised controlled Phase I/IIb trial. Lancet. 2007;370:1543-51.

27. Asante KP, Abdulla S, Agnandji S, Lyimo J, Vekemans J, Soulanoudjingar S, et al. Safety and efficacy of the RTS,S/AS01E candidate malaria vaccine given with expanded-programme-on-immunisation vaccines: 19 month follow-up of a randomised, open-label, phase 2 trial. Lancet Infect Dis. 2011 Oct;11(10):741-9.

28. Genton B, D'Acremont V, Lurati-Ruiz F, Verhage D, Audran R, Hermsen C, et al. Randomized double-blind controlled Phase I/IIa trial to assess the efficacy of malaria vaccine PfCS102 to protect against challenge with *P. falciparum*. Vaccine. 2010;28(40):6573-80.

29. Walther M, Thompson FM, Dunachie S, Keating S, Todryk S, Berthoud T, et al. Safety, immunogenicity, and efficacy of prime-boost immunization with recombinant poxvirus FP9 and modified vaccinia virus Ankara encoding the full-length *Plasmodium falciparum* circumsporozoite protein. Infect Immun. 2006;74:2706-16.

30. Walther M, Dunachie S, Keating S, Vuola JM, Berthoud T, Schmidt A, et al. Safety, immunogenicity and efficacy of a pre-erythrocytic malaria candidate vaccine, ICC-1132 formulated in Seppic ISA 720. Vaccine. 2005;23:857-64.

31. Kubler-Kielb J, Majadly F, Biesova Z, Mocca CP, Guo C, Nussenzweig R, et al. A bicomponent *Plasmodium falciparum* investigational vaccine composed of protein-peptide conjugates. Proc Natl Acad Sci U S A. 2010;107:1172-7.

32. Hoffman SL, Billingsley PF, James E, Richman A, Loyevsky M, Li T, et al. Development of a metabolically active, non-replicating sporozoite vaccine to prevent *Plasmodium falciparum* malaria. Hum Vaccin. 2010;6:97-106.

33. Vaughan AM, Wang R, Kappe SH. Genetically engineered, attenuated whole-cell vaccine approaches for malaria. Hum Vaccin. 2010;6: 107-13.

34. Hill AV, Reyes-Sandoval A, O'Hara G, Ewer K, Lawrie A, Goodman A, et al. Prime-boost vectored malaria vaccines: progress and prospects. Hum Vaccin. 2010;6:78-83.

35. Clyde DF. Immunity to falciparum and vivax malaria induced by irradiated sporozoites: a review of the University of Maryland studies, 1971–75. Bull World Health Organ. 1990;68 Suppl:9-12.

36. Hoffman SL, Goh LM, Luke TC, Schneider I, Le TP, Doolan DL, et al. Protection of humans against malaria by immunization with radiation-attenuated *Plasmodium falciparum* sporozoites. J Infect Dis. 2002;185:1155-64.

37. Epstein JE, Tewari K, Lyke KE, Sim BKL, Billingsley PF, Laurens MB, et al. Live attenuated malaria vaccine designed to protect through hepatic CD8+ T cell immunity. Science [Internet]. 2011 Sep 8. [Epub ahead of print]. Available from: www.sciencemag.org/content/early/2011/09/06/science.1211548.

38. Trimnell A, Takagi A, Gupta M, Richie TL, Kappe SH, Wang R. Genetically attenuated parasite vaccines induce contact-dependent CD8+ T cell killing of *Plasmodium yoelii* liver stage-infected hepatocytes. J Immunol. 2009;183:5870-8.

39. Webster DP, Dunachie S, Vuola JM, Berthoud T, Keating S, Laidlaw SM, et al. Enhanced T cell-mediated protection against malaria in human challenges by using the recombinant poxviruses FP9 and modified vaccinia virus Ankara. Proc Natl Acad Sci U S A. 2005;102:4836-41.

40. Bejon P, Mwacharo J, Kai O, Mwangi T, Milligan P, Todryk S, et al. A Phase 2b randomised trial of the candidate malaria vaccines FP9 ME-TRAP and MVA ME-TRAP among children in Kenya. PLoS Clin Trials. 2006;1:e29.

41. Fairley NH. Sidelights on malaria in man obtained by subinoculation experiments. Trans R Soc Trop Med Hyg. 1947;40:621-76.

42. Cohen S, McGregor GI, Carrington S. Gamma-globulin and acquired immunity to human malaria. Nature. 1961;192:733-7.

43. Sabchareon A, Burnouf T, Ouattara D, Attanath P, Bouharoun-Tayoun H, Chantavanich P, et al. Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. Am J Trop Med Hyg. 1991;45:297-308.

44. Sagara I, Dicko A, Ellis RD, Fay MP, Diawara SI, Assadou MH, et al. A randomized controlled Phase 2 trial of the blood stage AMA1-C1/Alhydrogel malaria vaccine in children in Mali. Vaccine. 2009;27:3090-8.

45. El Sahly HM, Patel SM, Atmar RL, Lanford TA, Dube T, Thompson D, et al. Safety and immunogenicity of a recombinant nonglycosylated erythrocyte binding antigen 175 region II malaria vaccine in healthy adults living in an area where malaria is not endemic. Clin Vaccine Immunol. 2010;17:1552-9.

46. Esen M, Kremsner PG, Schleucher R, Gassler M, Imoukhuede EB, Imbault N, et al. Safety and immunogenicity of GMZ2—a MSP3-GLURP fusion protein malaria vaccine candidate. Vaccine. 2009;27:6862-8.

47. Hermsen CC, Verhage DF, Telgt DS, Teelen K, Bousema JT, Roestenberg M, et al. Glutamate-rich protein (GLURP) induces antibodies that inhibit in vitro growth of *Plasmodium falciparum* in a Phase 1 malaria vaccine trial. Vaccine. 2007;25:2930-40.

48. Ogutu BR, Apollo OJ, McKinney D, Okoth W, Siangla J, Dubovsky F, et al. Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in Western Kenya. PLoS One. 2009;4:e4708.

49. Genton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, et al. A recombinant blood-stage malaria vaccine reduces *Plasmodium falciparum* density and exerts selective pressure on parasite populations in a Phase 1-2b trial in Papua New Guinea. J Infect Dis. 2002;185:820-7.

50. Audran R, Cachat M, Lurati F, Soe S, Leroy O, Corradin G, et al. Phase I malaria vaccine trial with a long synthetic peptide derived from the merozoite surface protein 3 antigen. Infect Immun. 2005;73:8017-26.

51. Sirima SB, Tiono AB, Ouedraogo A, Diarra A, Ouedraogo AL, Yaro JB, et al. Safety and immunogenicity of the malaria vaccine candidate MSP3 long synthetic peptide in 12–24 months-old Burkinabe children. PLoS One. 2009;4:e7549.

52. Druilhe P, Spertini F, Soesoe D, Corradin G, Mejia P, Singh S, et al. A malaria vaccine that elicits in humans antibodies able to kill *Plasmodium falciparum*. PLoS Med. 2005;2:e344.

53. Horii T, Shirai H, Jie L, Ishii KJ, Palacpac NQ, Tougan T, et al. Evidences of protection against blood-stage infection of *Plasmodium falciparum* by the novel protein vaccine SE36. Parasitol Int. 2010;59:380-6.

54. Ellis RD, Martin LB, Shaffer D, Long CA, Miura K, Fay MP, et al. Phase 1 trial of the *Plasmodium falciparum* blood stage vaccine MSP1(42)-C1/Alhydrogel with and without CPG 7909 in malaria naive adults. PLoS One. 2010;5:e8787.

55. Sagara I, Ellis RD, Dicko A, Niambele MB, Kamate B, Guindo O, et al. A randomized and controlled Phase 1 study of the safety and immunogenicity of the AMA1-C1/Alhydrogel + CPG 7909 vaccine for *Plasmodium falciparum* malaria in semi-immune Malian adults. Vaccine. 2009;27:7292-8.

56. Malkin E, Hu J, Li Z, Chen Z, Bi X, Reed Z, et al. A Phase 1 trial of PfCP2.9: an AMA1/MSP1 chimeric recombinant protein vaccine for *Plasmodium falciparum* malaria. Vaccine. 2008;26:6864-73.

57. Weedall GD, Conway DJ. Detecting signatures of balancing selection to identify targets of anti-parasite immunity. Trends Parasitol. 2010;26:363-9.

58. Takala SL, Plowe CV. Genetic diversity and malaria vaccine design, testing and efficacy: preventing and overcoming 'vaccine resistant malaria.' Parasite Immunol. 2009;31:560-73.

59. Takala SL, Coulibaly D, Thera MA, Batchelor AH, Cummings MP, Escalante AA, et al. Extreme polymorphism in a vaccine antigen and risk of clinical malaria: implications for vaccine development. Sci Transl Med. 2009;1:2ra5.

60. Moorthy V, Reed Z, Smith PG; WHO Study Group on Measures of Malaria Vaccine Efficacy. Measurement of malaria vaccine efficacy in Phase III trials: report of a WHO consultation. Vaccine. 2007; 25:5115-23.

61. Carter R, Mendis KN, Miller LH, Molineaux L, Saul A. Malaria transmission-blocking vaccines—how can their development be supported? Nat Med. 2000;6:241-4.

62. Kaslow DC, Quakyi IA, Keister DB. Minimal variation in a vaccine candidate from the sexual stage of *Plasmodium falciparum*. Mol Biochem Parasitol. 1989;32:101-3.

63. Chowdhury DR, Angov E, Kariuki T, Kumar N. A potent malaria transmission blocking vaccine based on codon harmonized full length Pfs48/45 expressed in *Escherichia coli*. PLoS One. 2009;4:e6352.

64. Quakyi IA, Carter R, Rener J, Kumar N, Good MF, Miller LH. The 230-kDa gamete surface protein of *Plasmodium falciparum* is also a target for transmission-blocking antibodies. J Immunol. 1987;139:4213-7.

65. Liu Y, Tewari R, Ning J, Blagborough AM, Garbom S, Pei J, et al. The conserved plant sterility gene HAP2 functions after attachment of fusogenic membranes in *Chlamydomonas* and *Plasmodium* gametes. Genes Dev. 2008;22:1051-68.

66. Blagborough AM, Sinden RE. Plasmodium berghei HAP2 induces strong malaria transmission-blocking immunity in vivo and in vitro. Vaccine. 2009;27:5187-94.

67. Saul A. Efficacy model for mosquito stage transmission blocking vaccines for malaria. Parasitology. 2008;135:1497-1506.

68. Wu Y, Przysiecki C, Flanagan E, Bello-Irizarry SN, Ionescu R, Muratova O, et al. Sustained high-titer antibody responses induced by conjugating a malarial vaccine candidate to outer-membrane protein complex. Proc Natl Acad Sci U S A. 2006;103:18243-8.

Respiratory Syncytial Virus

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Respiratory syncytial virus (RSV) is the single most important cause of severe lower respiratory tract infection in infants and young children. RSV disease also affects the elderly and the immunocompromised. It is a frequent cause of winter outbreaks of acute respiratory disease. RSV infects repeatedly and causes disease throughout life, including a wide array of respiratory symptoms, from rhinitis and otitis media to pneumonia and bronchiolitis—of which the latter two have significant morbidity and mortality. In the United States, 3.5–4 million children younger than 4 years of age acquire RSV infection annually. Among infants less than 1 year of age, RSV accounts for an estimated 75,000–125,000 hospitalizations annually. RSV infects nearly all children by 2 years of age, and re-infections occur later during childhood and adulthood that are generally associated with milder disease. Recent evidence points to a link between RSV infection and the development of wheezing and asthma [1].

Recently, RSV has been recognized as a significant cause of severe respiratory infections in older populations. Among the elderly in the United States, RSV accounts for an estimated 14,000–62,000 hospitalizations annually. Outbreaks of RSV are complicated with pneumonia among elderly patients in nursing homes and hospitals. Each year, RSV affects 5–10 percent of nursing home populations. Two to 8 percent of these cases are fatal, amounting to approximately 10,000 deaths per year

IMPACT OF REGULATORY SCIENCE ON INFLUENZA VACCINE DEVELOPMENT

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The Food and Drug Administration (FDA) pursues and promotes advances in regulatory science—the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of FDA-regulated products. The agency's Center for Biologics Evaluation and Research (CBER) regulates complex and diverse products, including vaccines intended to protect against both seasonal and pandemic influenza.

As part of its efforts to advance regulatory science, CBER plays a pivotal role in the development of tests that ensure the potency of seasonal and pandemic strain–specific influenza vaccines. Antibodies against the hemagglutinin (HA) protein from the influenza virus strain(s) that will be included in the vaccine are essential to testing the potency of the vaccine. CBER scientists typically remove the HA protein from influenza viruses using a standard chemical technique; these proteins are injected into sheep, whose immune systems make anti-HA antibodies. CBER collects the sheep sera containing these antibodies and supplies the sera for use in potency tests for influenza vaccines.

Although this approach to developing anti-HA antibodies is typically effective, there have been instances in which the peculiar characteristics of some strains of influenza virus make it difficult to obtain sufficient amounts of HA protein. Therefore, CBER developed an alternative approach that does not require the presence or purification of influenza virus or removal of HA protein. Instead, the center uses recombinant DNA techniques to produce plasmid DNA coding for HA protein and injects the plasmid into sheep. The HA protein expressed *in vivo* from this DNA triggers development of antibodies against the specific HA protein. CBER scientists then inject into the sheep genetically engineered viral vectors that produce HA protein to boost antibody production. These sheep anti-HA antibodies have worked effectively in tests designed to evaluate commercially produced H1N1 and H5N1 vaccines.

This work demonstrates the feasibility of an alternative approach to producing potency reagents [1] and provides an effective backup technique for anti-HA antibody production when the standard technique does not work well or fast enough to produce potency antibodies for a novel influenza virus. It is an example of the critical role CBER research plays in ensuring the safety, purity, potency, and effectiveness of biological products through regulatory science.

REFERENCE

1. Schmeisser, F, Vodeiko, GM, Lugovtsev, VY, Stout, RR, Weir JP. An alternative method for preparation of pandemic influenza strain-specific antibody for vaccine potency determination. Vaccines. 2010;28:2442-9.

from RSV among persons older than 64 years of age. Among elderly persons followed for three consecutive winters, RSV infection accounted for 11.4 percent of hospitalizations for obstructive pulmonary disease, 10.6 percent of hospitalizations for pneumonia, 7.2 percent of hospitalizations for asthma, and 5.4 percent of hospitalizations for congestive heart failure [2]. Severe RSV infections are also a problem in immunocompromised persons of any age, especially transplant recipients.

An effective vaccine to prevent RSV could be useful in reducing morbidity, the frequency of hospitalizations, and the death rate from this infection. Although the development of a vaccine has been a priority of NIAID, a licensed vaccine is not yet available because of several challenges. The most significant obstacle is the unexpected enhancement of disease post-vaccination (i.e., increased severity of infection when vaccinated children were exposed to natural RSV infection). In a study conducted in the 1960s, immunized children who were seronegative for RSV before vaccination and were subsequently exposed naturally to RSV experienced enhanced disease. This included a significant increase in the frequency and severity of RSV lower respiratory tract diseases (bronchoconstriction and pneumonia) and greater incidence of hospitalization, compared with children in the control group who were not vaccinated [3]. Scientists are studying possible mechanisms responsible for this enhanced disease following vaccination.

To develop an effective vaccine, a more complete understanding of the protective and disease-enhancing immune responses to RSV is imperative. Research efforts have focused on the individual components of these responses, including cell-mediated events and production of serum and secretory antibodies. Vaccine candidates under development are evaluated in a stepwise progression: first in animal models, next in adults, then in children—those who have already been exposed to infections (seropositive individuals), older nonimmune or seronegative children, and younger seronegative and highly susceptible infants.

RSV includes two subgroups: A and B. A successful vaccine would induce resistance to both of these subgroups. The major protective antigens of RSV are the fusion (F) and attachment (G) glycoproteins found on the surface of RSV. These proteins induce neutralizing antibodies that protect against wild-type RSV infection. The F surface protein is highly conserved among the RSV subgroups and functions to promote fusion of the virus and host-cell membranes. The structure of the G surface protein is the major difference between RSV subgroups A and B. The G protein is responsible for attaching RSV to a susceptible cell. Despite 47 percent amino acid sequence diversity between the G proteins in RSV subgroups A and B, the G protein contains a central conserved domain that is flanked by two hypervariable regions.

Subunit RSV Vaccine Candidates

Several potential vaccine candidates contain purified F protein (PFP). PFP–1 and PFP–2 are subunit vaccines that were tested in various populations in Phases I and II human clinical trials. In studies with 12- to 48-month-old RSV seropositive children, PFP–1 and PFP–2 have been shown to be safe and immunogenic. These studies were not designed to evaluate the efficacy of the vaccine (i.e., whether recipients are actually protected from RSV infection) [4].

Subunit vaccines may be particularly useful in specific groups of high-risk children and adults. In a pilot study of children with cystic fibrosis, the PFP–2 vaccine induced a significant antibody response and a significant reduction in the number of lower respiratory tract illnesses [5]. Other studies have demonstrated that the PFP–2 vaccine is safe and immunogenic in ambulatory adults older than 60 years of age and in seropositive children who have bronchopulmonary dysplasia [6, 7].

A Phase II, double-blind, controlled, multicenter study of the safety, immunogenicity, and effectiveness of the PFP–3 subunit vaccine was conducted in RSV seropositive children with cystic fibrosis. The study found that the PFP–3 subunit vaccine is safe and immunogenic; however, the study did not demonstrate a reduction in the incidence of lower respiratory tract illnesses [8].

Maternal immunization with a PFP subunit vaccine is a potential strategy being evaluated to protect infants younger than 6 months old from RSV disease. The rationale is based on (1) reports of the efficient transfer of specific neutralizing antibodies from mothers to infants during pregnancy and (2) demonstration of the possible prophylactic value of high-titer anti-RSV polyclonal antiserum or humanized monoclonal antibody (MAb) that is administered to high-risk children to protect against lower respiratory tract RSV disease and hospitalization [9]. Infants younger than 6 months old are most at risk for RSV infection, but usually least responsive to vaccines. Thus, maternal immunization may be beneficial because pregnant women respond well immunologically to vaccines and placental transfer of maternal antibodies occurs naturally during the third trimester. A Phase I, double-blind, placebo-controlled study was conducted with 35 healthy women who were in their third trimester of pregnancy. The

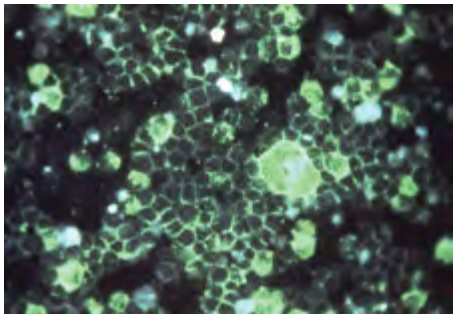
PFP–2 vaccine was found to be safe and immunogenic. Transplacental transfer of maternal neutralizing antibodies to RSV was efficient. Infants born to vaccine recipients were healthy and did not experience adverse events related to maternal immunization [9].

The G protein fragment of RSV is the basis of another subunit vaccine currently being developed. A novel recombinant vaccine candidate, BBG2Na, has been constructed by fusing the conserved central domain of the G protein (G2Na) of RSV Long strain to BB (the albumin-binding region of streptococcal G protein). A clinical trial was conducted in 108 healthy adults. The BBG2Na vaccine was found to be safe, well-tolerated, and immunogenic [10].

A subunit RSV vaccine consisting of F, G, and M proteins also is being developed. Little is known about the function of the M protein (Matrix protein), but some data suggest that the M protein is associated with RSV nucleocapsids and, like the matrix proteins of other negative-strand RNA viruses, can inhibit virus transcription. The primary target of this vaccine is to prevent significant respiratory disease in study populations primed by previous natural RSV infection. Two Phase I clinical trials have been conducted in healthy adults. These trials support the safety and immunogenicity of this product. The first trial compared an aluminum phosphate formulation of the vaccine (n=30) with aluminum phosphate control (n=10). The second trial compared the aluminum phosphate formulation (n=10) with a formulation containing a new adjuvant—poly[di(carboxylatophenoxy)phosphazene]—in a different sample of young, healthy adults (n=30). Both vaccines were found to be well-tolerated and immunogenic [11]. Additional studies of the F/G/M protein vaccine are being conducted.

Several other subunit vaccines are in preclinical development:

- Recombinant chimeric RSV FG glycoprotein vaccines adsorbed onto aluminum hydroxide gel with or without the addition of 3-deacylated monophosphoryl lipid A
- PFP formulated with alum with or without G protein (from subgroups A and B)
- Synthetic peptide of the conserved region of the G protein with or without cholera toxin as a mucosal adjuvant
- Recombinant fragment (BBG2Na) of the G protein formulated with dimethyldioctadecylammonium bromide, a nasal adjuvant
- Recombinant fragment of the G protein in a liposome encapsulated formulation, prepared by including a variety of different lipids



Photomicrographic detection of respiratory syncytial virus (RSV) using indirect immunofluorescence technique. Courtesy of CDC

- Mimotope (peptide that mimics antigenicity) of a conserved and conformationally determined epitope of the F protein recognized by an anti-RSV MAb (MAb19) that neutralizes RSV
- Recombinant RSV F virus-like particles
- Recombinant RSV F and G proteins using Newcastle disease virus-like particles
- Recombinant F and G proteins using Sendai virus as a vector

Live Attenuated RSV Vaccine Candidates

NIAID laboratories are actively pursuing the development of a live attenuated RSV vaccine that is administered intranasally. Live attenuated vaccines appear to offer several advantages over nonreplicating or subunit vaccines, especially for RSV-naive infants and young children. Intranasal immunization with a live attenuated vaccine induces both systemic and local immunity and therefore may protect against upper as well as lower respiratory disease. Also, the immune response to a live vaccine more closely resembles the response to natural infection and therefore is less likely to produce enhanced disease on exposure to natural infection. In addition, like other live attenuated intranasal respiratory virus vaccine candidates, live intranasal RSV vaccine candidates have been shown to replicate in young infants in the presence of maternally acquired antibodies.

Early attempts at developing live attenuated RSV strains included conventional methods of attenuation by cold passage (cp), cold adaptation, chemical mutagenesis, temperature-sensitive (ts) selection, and combinations of these methods. These efforts resulted in several vaccine candidates that appeared to be substantially attenuated in experimental animals. These candidates were then evaluated in Phase I clinical studies, which involve a stepwise progression from adults to seropositive children to seronegative children to RSV-naive infants. These viruses proved to be insufficiently attenuated. The most promising candidate was a cold-passaged,

temperature-sensitive mutant called cpts248/404, which was well-tolerated and immunogenic in seronegative children older than 6 months of age. However, cpts248/404 was associated with mild-to-moderate upper respiratory congestion when administered to 1- to 2-month-old infants, indicating that more attenuation was needed [12].

To construct more-attenuated vaccine candidates, the technology of reverse genetics was employed, whereby complete infectious RSV is recovered from cDNA. This provides the means to insert predetermined mutations into infectious viruses via the cDNA intermediate. This technique was coupled with sequence analysis to determine the basis of attenuation in the incompletely attenuated, biologically derived viruses noted above. This resulted in identification of the mutations involved in the attenuated cp phenotype and of six independent ts-attenuating mutations. In addition, four accessory viral genes were identified (SH, NS1, NS2, and M2–2) that are nonessential in cell culture but are attenuating *in vivo;* thus, deleting these genes provides another means of attenuation. With this information, a series of further-attenuated, cDNA-derived viruses were constructed. In particular, a recombinant version of cpts248/404 (the mutant described above) was further attenuated by deleting the SH gene and including yet another attenuating mutation, yielding a virus called cp248/404/1030ΔSH. When evaluated in 4- to 12-week-old infants, this virus was well-tolerated and immunogenic [13]. Additional studies are needed to determine whether cp248/404/1030ΔSH can induce protective immunity against wild-type RSV.

Other candidates are being prepared for clinical studies. Deleting the M2–2 coding sequence resulted in a virus that is reduced one-thousandfold for replication in experimental animals and has the unusual phenotype of decreased RNA replication and increased gene transcription and antigen expression. Another candidate that is presently being prepared for clinical evaluation involves deleting the NS1 gene, which was shown to strongly suppress the induction of type I interferon. Both the delM2–2 and delNS1 viruses may have increased immunogenicity due to, respectively, increased antigen expression and the adjuvant effect of increased interferon expression. Additional candidates involving combinations of gene deletions and point mutations designed to increase genetic stability also are being developed. The vaccine candidates to date represent RSV antigenic subgroup A; a subgroup B component also will likely be included in an RSV vaccine, which can be readily achieved using the same attenuating mutations that have been identified for subgroup A.

Another strategy is to express the RSV F and G protective antigens from genes added to a live human parainfluenza virus type 3 (HPIV3) vaccine as vector. HPIV3 is a particularly apt choice, because immunization against both RSV and HPIV3 ideally should begin early in infancy. Presently, lead constructs have been developed based on an attenuated PIV3 consisting of bovine PIV3 in which the F and HN genes have been replaced by those of HPIV3, thus combining the host-range attenuation of bovine PIV3 with the major protective antigen genes from HPIV3. A construct in which the RSV F protein is expressed from an added gene between the N and P genes of the PIV3 vector is currently in Phase I clinical trials. On one hand, this approach combines two necessary vaccines into a single recombinant virus and, being based on PIV3, avoids the poor growth and physical instability of RSV. But on the other hand, the construct lacks most of the RSV antigens. Combining a PIV-vectored RSV vaccine with an attenuated RSV strain may be the best way to increase the potency of immunization against RSV while including a PIV3 component.

The live attenuated approach was evaluated in healthy young adults, showing that these viruses are highly restricted and over-attenuated in RSV-experienced individuals [14]. The live attenuated approach will likely not be useful in adults because a virus that replicates well in RSV-experienced individuals likely will retain residual virulence for RSV-naive contacts. However, RSV subunit vaccines have been shown to be well-tolerated and safe in RSV-experienced individuals, which is consistent with the observation that, to date, disease enhancement has been observed in only RSV-naive individuals [3]. The immunogenicity of previous formulations of RSV subunit vaccines was disappointing, but several commercial companies are developing improved versions. An RSV subunit vaccine could be combined with the inactivated influenza vaccine for yearly immunization. Maternal immunization with an inactivated vaccine represents another possible approach to increasing the resistance of young infants to severe RSV disease.

Future Directions

Ideally, immunization for RSV should begin during the first 2 months of life. However, developing a vaccine for RSV is challenging because this is a time when immune responses are reduced due to immunologic immaturity and the presence of maternal antibodies. Safety concerns also are paramount during this time. In addition, RSV infects and causes disease

at the lung mucosal surface, where immune protection is less complete. However, the recent success of the live attenuated, topically administered rotavirus vaccine indicates that substantial reduction in severe disease from a mucosal pathogen can be achieved in infancy [15]. While still elusive, live attenuated RSV vaccine candidates with promising characteristics are now moving into expanded clinical trials. The development of improved subunit vaccines has great potential for use in healthy adults, the elderly, and specific groups of high-risk older children, as well as for maternal immunization.

In addition, substantial progress has been made in developing new adjuvants for human use. These adjuvants may augment the immunogenicity of subunit vaccines and possibly live vaccines. With appropriate adjuvants, RSV subunit vaccines might be made safe for RSV-naive individuals. New RSV vaccine platforms, including virus-like particles and replication-defective vectors such as alphaviruses and adenoviruses, have yielded promising results in preclinical testing. Thus, the prospects for developing RSV vaccines are encouraging.

REFERENCES

1. Henderson J, Hilliard TN, Sherriff A, Stalker D, Al Shammari N, Thomas HM. Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. Pediatr Allergy Immunol. 2005 Aug;16(5):386-92.
2. World Health Organization (WHO). Acute respiratory infections (Update September 2009) [Internet]. Geneva, Switzerland: WHO; 2009. Disease burden, RSV infection. Available from: www.who.int/vaccine_research/diseases/ari/en/index2.html
3. Fulginiti VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, Meiklejohn G. Respiratory virus immunization. A field trial of two inactivated respiratory virus vaccines; an aqueous trivalent parainfluenza virus vaccine and an alum-precipitated respiratory syncytial virus vaccine. Am J Epidemiol. 1969 Apr;89(4):435-48.
4. Durbin AP, Karron RA. Progress in the development of respiratory syncytial virus and parainfluenza virus vaccines. Clin Infect Dis. 2003 Dec 15;37(12):1668-77. Epub 2003 Nov 20.
5. Piedra PA, Grace S, Jewell A, Spinelli S, Bunting D, Hogerman DA, et al. Purified fusion protein vaccine protects against lower respiratory tract illness during respiratory syncytial virus season in children with cystic fibrosis. Pediatr Infect Dis J. 1996 Jan;15(1):23-31.
6. Falsey AR, Walsh EE. Safety and immunogenicity of a respiratory syncytial virus subunit vaccine (PFP–2) in ambulatory adults over age 60. Vaccine. 1996 Sep;14(13):1214-8.
7. Groothuis JR, King SJ, Hogerman DA, Paradiso PR, Simoes EA. Safety and immunogenicity of a purified F protein respiratory syncytial virus (PFP–2) vaccine in seropositive children with bronchopulmonary dysplasia. J Infect Dis. 1998 Feb;177(2):467-9.
8. Piedra PA, Grace S, Jewell A, Spinelli S, Hogerman DA, Malinoski F, et al. Sequential annual administration of purified fusion protein vaccine against respiratory syncytial virus in children with cystic fibrosis. Pediatr Infect Dis J. 1998 Mar;17(3):217-24.
9. Munoz FM, Piedra PA, Glezen WP. Safety and immunogenicity of respiratory syncytial virus purified fusion protein-2 vaccine in pregnant women. Vaccine. 2003 Jul 28;21(24):3465-7.
10. Goetsch L, Plotnicky-Gilquin H, Aubry JP, De-Lys P, Haeuw JF, Bonnefoy JY, et al. BBG2Na an RSV subunit vaccine candidate intramuscularly injected to human confers protection against viral challenge after nasal immunization in mice. Vaccine. 2001 Jul 16;19(28-29):4036-42.
11. Sales V, Goldwater R, Warren JT, Yi Q, Caudrelier P. Safety and immunogenicity of a respiratory syncytial virus subtype A vaccine in adults: two Phase I studies. Presented at: IV International Symposium on Respiratory Viral Infections; 2001 Dec; Curacao, Netherlands Antilles.
12. Wright PF, Karron RA, Belshe RB, Thompson J, Crowe JE Jr, Boyce TG, et al. Evaluation of a live, cold-passaged, temperature-sensitive, respiratory syncytial virus vaccine candidate in infancy. J Infect Dis. 2000 Nov;182(5):1331-42. Epub 2000 Sep 22.
13. Karron RA, Wright PF, Belshe RB, Thumar B, Casey R, Newman F, et al. Identification of a recombinant live-attenuated respiratory syncytial virus vaccine candidate that is highly attenuated in infants. J Infect Dis. 2005;191:1093-1104.
14. Friedewald WT, Forsyth BR, Smith CB, Gharpure MA, Chanock RM. Low-temperature-grown RS virus in adult volunteers. JAMA. 1968 May 20;204(8):690-4.
15. Linhares AC, Velázquez FR, Pérez-Schael I, Sáez-Llorens X, Abate H, Espinoza F, et al.; Human Rotavirus Vaccine Study Group. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled Phase III study. Lancet. 2008 Apr 5;371(9619):1181-9.

Tuberculosis

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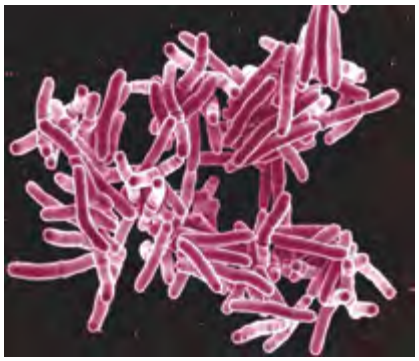
Background

Despite significant advances in tuberculosis (TB) research and improvement in treatment strategies, TB remains one of the leading infectious killers worldwide. Although curable, TB claims an estimated 1.7 million lives each year [1]. Failure to contain this disease can be attributed to a number of factors, including insufficient TB treatment and care infrastructure in endemic, resource-limited countries; the lack of integration of TB and HIV/AIDS healthcare services in areas where the spread of TB is closely linked to the HIV co-epidemic; the lack of rapid and sensitive diagnostics; the lack of treatment options to shorten therapy from the current 6–9 months; the spread of drug-resistant disease; and the lack of a highly effective vaccine.



Scanning electron micrograph of *Mycobacterium tuberculosis*. Courtesy of NIAID

In most cases, infection with *Mycobacterium tuberculosis (Mtb)* is controlled by the immune system and leads to a spectrum of manifestations ranging from asymptomatic colonization (often referred to as latent or persistent infection) to subclinical disease. Weakening of the immune system, as is the case in persons also infected with HIV or with diabetes, can result in progression from subclinical infection to active, symptomatic TB disease. While TB can manifest itself in a multitude of forms, pulmonary disease is of greatest public health importance since it is responsible for the transmission of the pathogen in communities. Patients with active TB are generally treated with combination chemotherapy under direct observation (DOT, directly observed treatment) for 6–9 months. The length of this regimen, combined with drug-related adverse events, frequently leads to noncompliance and treatment failures, which in turn can result in the development and spread of drug-resistant TB. According to modeling studies, a combination of prevention strategies using more effective vaccines and/or more efficient treatment of latent disease, combined with proactive identification and treatment of TB patients, are needed to eliminate this disease as a global public health burden [2].

The currently available TB vaccine, *M. bovis* Bacille Calmette-Guérin (BCG), was developed almost 100 years ago. Worldwide, a variety of BCG strains are available and are widely administered to newborn children under the World Health Organization's (WHO's) Expanded Programme on Immunization. One BCG vaccine strain (Tice) is licensed in the United States against TB but is not recommended for general use. Despite its lack of consistent, reproducible efficacy in clinical trials to prevent adult pulmonary TB, BCG provides reasonable protection against childhood complications of and death from TB.

Development of more effective vaccines either to prevent infection with *Mtb* or to block progression to active disease remains a priority for the National Institute of Allergy and Infectious Diseases (NIAID). Since 1998, when the U.S. Department of Health and Human Services' (HHS') Advisory Council for Elimination of Tuberculosis, the U.S. National Vaccine Program Office, and NIAID convened a workshop to develop the Blueprint for Tuberculosis Vaccine Development, several promising vaccine candidates have become available, many of which are now being evaluated in humans in clinical trials.

State of the Science in Tuberculosis Vaccine Development

Until the early 1980s, the incidence of TB in the United States had been steadily declining. A sudden spike in new cases was reported between 1986 and 1992. This resurgence of TB was attributable largely to a deteriorating public health infrastructure and also was coincident with the HIV epidemic. In 1993, TB was declared a global health emergency by WHO [3]. Following these events, awareness of the global impact of TB increased and led to the realization that improving our understanding of the natural history of TB and the interaction of host and pathogen is a prerequisite for identifying better ways to diagnose, prevent, and treat this disease. Research funding

has steadily increased since 1992, with NIAID developing a comprehensive research program to stimulate and support all aspects of TB biomedical research and product development. Significant gains in knowledge were made through the sequencing of the genome of laboratory and clinical strains of *Mtb* and other mycobacterial species, and the development of microbiologic and genetic tools that helped dissect the interaction of the pathogen and the host immune response. These efforts have been aided by the development of research resources for TB, including structural genomics consortia and collection of data using a systems biology approach—activities that have been funded by NIAID and through National Institutes of Health (NIH)-wide initiatives. These investments in biomedical research have resulted in the first-ever portfolio of TB vaccine candidates, many of which have entered clinical trials, with others completing preclinical evaluations. These candidates are representatives of a diverse set of vaccine classes and include recombinant BCG and live-attenuated *Mtb* strains; various other live vectors (bacterial and viral); and DNA, protein, and peptide subunit vaccines.

Significant effort has been expended to develop relevant animal models of TB that approximate distinct stages of human disease, to aid in the characterization and selection of preclinical and clinical vaccine candidates. Since the pathogenesis of TB varies among different animal species, with dynamic immunological factors modulating disease outcome after infection with *Mtb*, several different animal species are currently employed in preclinical vaccine testing, to assemble comprehensive datasets about vaccine candidates. Through the increasingly detailed characterization and refinement of these models, which now extend from rodents (mice and guinea pigs) to rabbits to nonhuman primates, researchers continue to gain insight into immunological and microbiologic factors that are involved in the development of TB in these animals and thus create scientific hypotheses for how human TB may develop. Although it is recognized that BCG provides critical protection against pediatric TB, this live vaccine can lead to significant adverse events and even death in children also infected with HIV, and thus, safer and more effective versions of BCG are being developed. Clinical development strategies for new TB vaccines include boosting of neonatal BCG with novel vaccines at a later stage in life, as well as replacement of BCG with safer and more effective recombinant strains that will improve boosting later in life. Both strategies to prevent primary infection and/or reactivation of latent TB are being pursued, as are strategies to use vaccines and immune stimulants to improve and shorten chemotherapy. Because about one-third of the world's population is thought to harbor asymptomatic infection with *Mtb*, and HIV co-infection increases the chance of developing active disease from 1 in 10 over the course of a person's life to 1 in 10 per year, prevention of reactivation disease is considered critical to curbing the spread of TB [4].

Several candidates that demonstrated protection against infection with *Mtb* in small animal models equally well or better than BCG have entered human clinical trials. These are the first studies of new, engineered TB vaccine candidates since the introduction of BCG in 1921. This new generation of clinical candidates includes recombinant BCG vaccines expressing various immunodominant *Mtb* antigens intended to replace BCG as a primary vaccine and fusion proteins composed of immunogenic *Mtb* peptides and virally vectored constructs intended to boost either current or potential recombinant BCG. In addition, various non-TB mycobacteria, such as *M. vaccae* and *M. w,* are being evaluated for their ability to stimulate immune responses against TB. Also, clinical studies are being conducted to better define the immune protection elicited by BCG in pediatric populations and to aid in the development of immune assays for the characterization of immune responses in human clinical trials. Overall, the research community is developing a comprehensive approach to designing improved vaccination strategies for TB. Currently, it is estimated that combination approaches of improved priming and boosting vaccines will be needed to produce protective immune responses in adult populations.

Challenges and Opportunities for Developing a Vaccine for Tuberculosis

The majority of research toward new and improved vaccines has only occurred during the last decade. Hence, little historical experience in TB vaccinology is available that can be used as guidance for developing or improving new TB vaccines. Although TB vaccine research has made tremendous advances over the last 10 to 15 years, a number of critical questions remain to be answered. The answers will likely provide the keys to faster TB vaccine development.

- Why are some individuals able to contain infection with *Mtb* as a latent, asymptomatic infection while others develop subclinical disease and still others progress to fulminate active disease? To answer this question, longitudinal human studies of *Mtb* infection are needed to define approaches and solutions to preventing progression to active disease.

- What markers can serve as correlates of immunoprotection in humans to allow assessment of immunogenicity in clinical trials? Since BCG is not able to protect against adult TB, these correlates of immune protection will likely not be identified until vaccines that provide more effective protection are evaluated in advanced clinical trials. Research in immunology of TB has provided suggestions as to what markers may be of relevance in protection, and these markers are progressively being integrated into clinical immune assays and also in animal studies of TB vaccines. Only with the aid of data from human vaccine trials will researchers be able to benchmark animal models to help identify those candidate vaccines with the highest chance of improving protection against TB in humans. For these reasons, it is critical that vaccine candidates be quickly evaluated for safety and efficacy in human trials and any subsequent findings used to devise more targeted vaccine strategies.
- What is the importance of co-infections and comorbidities in patients at high risk for *Mtb* infection and progression to active disease? Do such co-infections or comorbidities have an impact on potential efficacy of vaccines?
- What are the most relevant animal models to predict efficacy of human vaccines against infection, disease, and/or transmission?
- How will persons already infected with *Mtb* respond to vaccination?
- What is the impact of vaccination on disease pathogenesis, and does natural and induced immunity affect the evolution of *Mtb* strain phenotypes? How do clinical trials have to be designed to study these complex interactions?
- What role will diagnostics play in the development of TB vaccines? Rapid and accurate identification of patients with *Mtb* infection, as well as ruling out active TB in adults and pediatric populations, will be critical for enrollment into clinical trials that evaluate post-exposure vaccines. Diagnostics that accurately and rapidly indentify infected persons are likely going to rely on a combination of host immune and bacterial markers. Diagnostic development therefore should be closely coupled with immunology and vaccinology research in TB to leverage scientific findings in these areas.
- How does BCG work in children? This is a currently understudied but important aspect of vaccine development. Little is known about general or TB-specific differences in immune response and vaccine efficacy among infants, children, and adults. It is recognized that the clinical presentation of TB in young children is different from that in adults and that BCG efficacy differs significantly in these populations.
- How can studies be designed to minimize the sample size and study duration? The current global capacity for registration-quality clinical trials for TB vaccines is insufficient to support Phase III trials. Furthermore, these trials are expected to require substantial numbers of trial volunteers and financial support, and it is unclear how development of clinical sites and funding for the clinical trials will be supported.

NIAID-Supported Tuberculosis Vaccine Research

Many challenges exist that will influence the design of efficacy trials in humans. To answer the above questions, NIAID is funding not only investigator-initiated research but also solicited research on TB immunology, pathology, pathogenesis, vaccine development, target antigen identification, diagnostics, development of improved tools for epidemiological studies, and development of markers of immunoprotection. All research in TB is included under Category C of NIAID's Biodefense Research Program. In addition, NIAID provides resources through its genomics and bioinformatics programs that are available to the TB research community.

NIAID's preclinical contract research resources include critical research materials from pathogenic and nonpathogenic mycobacteria, as well as vaccine-testing services in small animal models. Other contracts bridge the gap between identification of genes that may play a role in interaction between host and pathogen and actual determination of the biological function of these genes. Support services also are available to help advance promising preclinical candidates to clinical testing. NIAID's Tuberculosis Research Unit and Vaccine and Treatment Evaluation Units provide clinical trials infrastructure for TB projects to evaluate vaccine candidates and conduct studies on establishing surrogate markers of protection (see www.niaid.nih.gov/labsandresources/resources/Pages/default.aspx).

Knowledge gained from research over the last 14 years has led to a diverse pipeline of vaccine candidates, with several products being evaluated in various stages of clinical trials. The advancement of the current global TB vaccine pipeline, as well as an updated Blueprint for Tuberculosis Vaccine Development, is being discussed by members of the Stop TB Partnership's working group for vaccines. Its most recent publication, "The Global Plan to Stop TB 2011–2015," not only summarizes the ongoing efforts in the field of TB vaccine development but also,

for the first time in the history of TB control, acknowledges the need to include fundamental research in human TB as an integral part of a global strategy to eliminate this disease [5]. This publication attests to the continued need for new vaccines against TB and also recognizes the need for continued funding for and contributions from fundamental and translational science, both of which are heavily supported by NIAID. Although the field of TB vaccine development has produced a rich array of potential candidates and many donors are continuing to support preclinical research, a clear funding and "interest" gap continues to exist for pharmaceutical quality preclinical and also clinical development of vaccine candidates.

Despite the many challenges remaining in TB vaccine development, a new sense of optimism is permeating the TB research and public health communities, as recent research advances result in novel vaccine candidates entering human trials.

HEPATITIS C VIRUS: PROSPECTS FOR VACCINE DEVELOPMENT

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In the United States, there are approximately 20,000 new hepatitis C virus (HCV) infections every year. Acute HCV infections become chronic in the majority of infected individuals. Chronic HCV infection is associated with a high risk of progressive severe liver disease, including cirrhosis, liver cancer, and end-stage liver disease. There are an estimated 3–4 million individuals with chronic HCV infection in the United States and more than 170 million worldwide.

Multiple challenges exist with regard to developing an HCV vaccine. HCV mutates at an unusually high rate in an infected patient; immune responses such as virus-neutralizing antibodies and T-cell responses are compromised by the emergence of variant viruses. HCV proteins directly target and inhibit both innate and adaptive host immune responses. Also, a convenient small animal infection model for HCV is lacking. Currently, the only animal that can be infected with HCV is the chimpanzee.

The National Institute of Allergy and Infectious Diseases (NIAID) supports basic and clinical research on HCV replication and pathogenesis, virus-host interactions involved in pathogenesis, and immune responses; development of cell culture and small animal model systems for virus replication; development of vaccines and therapeutics, including programs to develop and test vaccines against HCV; and support of preclinical and clinical development resources.

Notably, in addition to individual investigator-initiated awards, NIAID has established five cooperative research centers for studying HCV, each engaged in studies on the host immunological response to infection.

Specific HCV vaccine candidates currently in clinical development include:

- A prime-boost approach with recombinant adenovirus and modified vaccinia Ankara (MVA) vectored vaccines preparing to enter Phase II trials
- Yeast vector vaccine in Phase IIb trial for therapeutic use
- Synthetic peptide vaccines—Phase II trials for therapeutic use completed
- MVA vector vaccine in Phase II trial for therapeutic use

The long-term, progressive clinical manifestations of chronic HCV infection provide opportunities, post-infection, to intervene with so-called "therapeutic" immunization approaches. Studies in chimpanzees suggest that it may be possible to develop both a prophylactic vaccine to prevent chronic HCV infection as well as therapeutic vaccines that may lower virus levels and ameliorate chronic liver disease. Given the large number of individuals with chronic HCV, safe and effective therapeutic vaccines that may potentially be used in conjunction with drugs would have great impact on the public health burden of HCV.

REFERENCES

1. World Health Organization. Tuberculosis [fact sheet on the Internet]. Geneva: World Health Organization; 2010 Nov. Available from: www.who.int/mediacentre/factsheets/fs104/en/index.html
2. Brewer T, Heymann S. To control and beyond: moving towards eliminating the global tuberculosis threat. J Epidemiol Community Health. 2004 Oct;58(10):822-5.
3. World Health Organization. 2007 global TB control report: epidemic levelling off [press release on the Internet]. Geneva: World Health Organization; 2007 Mar 22. Available from: www.who.int/tb/features_archive/wtbd07_press/en/index.html
4. Getahun H, Gunneberg C, Granich R, Nunn P. HIV infection-associated tuberculosis: the epidemiology and the response. Clin Infect Dis. 2010 May 15;50 Suppl 3:S201-7.
5. Stop TB Partnership. The global plan to stop TB 2011–2015 [report on the Internet]. Geneva: World Health Organization; 2011. Available from: www.stoptb.org/global/plan/

FURTHER READING

Aagaard C, Dietrich J, Doherty M, Andersen P. TB vaccines: current status and future perspectives. Immunol Cell Biol. 2009 May-Jun;87(4):279-86. Epub 2009 Apr 7.

Advisory Committee for the Elimination of Tuberculosis. The role of BCG vaccine in the prevention and control of tuberculosis in the United States. A joint statement by the Advisory Council for the Elimination of Tuberculosis and the Advisory Committee on Immunization Practices. MMWR Recomm Rep. 1996;45(RR-4):1-18.

Brennan MJ, Morris SL, Sizemore CF. Tuberculosis vaccine development: research, regulatory and clinical strategies. Expert Opin Biol Ther. 2004;4:1493-1504.

Centers for Disease Control and Prevention. Reported tuberculosis in the United States, 2009 [Surveillance Report on the Internet]. Atlanta (GA): Centers for Disease Control and Prevention. Available from: www.cdc.gov/tb/statistics/reports/2009/default.htm

Colditz GA, Berkey CS, Mosteller F, Brewer TF, Wilson ME, Burdick E, et al. The efficacy of bacillus Calmette-Guérin vaccination of newborns and infants in the prevention of tuberculosis: meta-analyses of the published literature. Pediatrics. 1995;96(1 Pt 1):29-35.

Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta analysis of the published literature. JAMA. 1994 Mar 2;271(9):698-702.

Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature. 1998;393:537-44. Erratum in: Nature. 1998;396:190.

Dye C, Williams BG. The population dynamics and control of tuberculosis. Science. 2010 May 14;328(5980):856-61.

Geiter L. Ending neglect: the elimination of tuberculosis in the United States. Washington, DC: Committee on the Elimination of Tuberculosis in the United States, Division of Health Promotion and Disease Prevention, Institute of Medicine; 2000.

Hanekom WA, Lawn SD, Dheda K, Whitelaw A. Tuberculosis research update. Trop Med Int Health. 2010 Aug;15(8):981-9. Epub 2010 Jun 15.

Mathema B, Kurepina N, Fallows D, Kreiswirth BN. Lessons from molecular epidemiology and comparative genomics. Semin Respir Crit Care Med. 29(5):467-80. Epub 2008 Sep 22.

McShane H. Need for more TB vaccine field sites. Indian J Exp Biol. 2009 Jun;47(6):445-6.

McShane H. Co-infection with HIV and TB: double trouble. Int J STD AIDS. 2005;16(2):95-100.

Minassian AM, McShane H. Tuberculosis vaccines: present and future. Expert Rev Respir Med. 2008 Dec;2(6):721-38.

National Institute of Allergy and Infectious Diseases. NIAID global health research plan for HIV/AIDS, malaria, and tuberculosis [Internet]. Bethesda (MD): National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Department of Health and Human Services; 2001 May 1. Available from: www.niaid.nih.gov/about/whoWeAre/Documents/global.pdf

National Institute of Allergy and Infectious Diseases. NIAID biodefense research agenda for category B and C priority pathogens [Internet]. Bethesda (MD): National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Department of Health and Human Services; 2003 Jan. Available from: www.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/about/Pages/strategicplan.aspx

National Institutes of Health. Estimates of funding for various research, condition, and disease categories (RCDC) [Internet]. Bethesda (MD): National Institutes of Health, U.S. Department of Health and Human Services; 2011 Feb 14. Available from: http://report.nih.gov/rcdc/categories/

Rouanet C, Locht C. Boosting BCG to protect against TB. Expert Rev Respir Med. 2010 Jun;4(3):339-48.

Thaiss CA, Kaufmann SHE. Toward novel vaccines against tuberculosis: current hopes and obstacles. Yale J Biol Med. 2010;83(4):209-15.

Wilson ME. Applying experiences from trials of Bacille Calmette-Guérin vaccine. Clin Infect Dis. 2000 Jun;30 Suppl 3:S262-5.

World Health Organization. Revised BCG vaccination guidelines for infants at risk for HIV infection. Wkly Epidemiol Rec. 2007;82(21):193-6.

World Health Organization. Global Tuberculosis Control 2010 [report on the Internet]. Geneva: World Health Organization; 2010. Available from: www.who.int/tb/publications/global_report/2010/en/index.html

Rotavirus Vaccines

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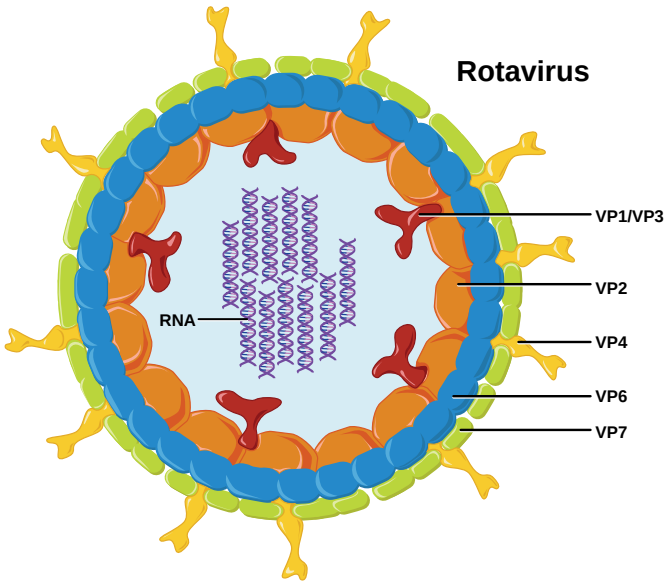
Rotaviruses are the leading cause of severe acute gastroenteritis among children around the world [1]. Before rotavirus vaccines were made available, nearly all children in the United States had rotavirus gastroenteritis by the age of 5, according to the Centers for Disease Control and Prevention (CDC). In the pre-vaccine era, rotavirus infections were responsible for 400,000 doctor visits, more than 200,000 emergency room visits, 55,000 hospitalizations, and 20 to 60 deaths annually among children under 5 years of age in the United States [2, 3].

Following the availability of rotavirus vaccines, reductions in severe and fatal diarrheal disease have been observed in low-middle, middle, and high-income countries [4]. It is estimated that in the United States rotavirus vaccines prevented approximately 650,000 diarrheal-associated hospitalizations between 2007 and 2009, and saved $278 million in treatment costs [5].

Vaccine-preventable deaths still continue, however. The World Health Organization estimates that more than 520,000 children under the age of 5 die from vaccine-preventable rotavirus infections each year, primarily in poor countries due to the lack of health care and adequate resources [1].

FIGURE 1.

Depiction of rotavirus



A rotavirus is a wheel-shaped virus consisting of 11 double-stranded RNA segments that generate six structural proteins (VP1, VP2, VP3, VP4, VP6, and VP7) and six nonstructural proteins (NSP1–6). Each virus particle is surrounded by a triple layer coat composed of the different structural proteins. Courtesy of NIAID

History of Rotavirus Vaccines

Credit for the discovery of human rotaviruses goes to Dr. Ruth Bishop in Melbourne, Australia, who first identified rotavirus as an agent of children's diarrhea in 1973. She recognized that naturally attenuated strains of rotavirus infecting neonates could protect them against severe gastroenteritis for multiple years.

Researchers later determined that rotaviruses consist of 11 segments of double-stranded RNA housed within concentric shells composed of three structural protein layers (Figure 1). There are seven rotavirus serogroups, A to G, with A being the most common. Proteins that form the outer shell include VP7 and the VP4 spike proteins. They stimulate the production of neutralizing antibodies and are, thus, targets for host protection by vaccines. VP6, which forms the next shell layer, has important antigenic determinants specific to each serogroup. One of the nonstructural proteins, NSP4, is now identified as an enterotoxin. When intestinal cells are infected with different strains of rotavirus—human or animal—genetic material from each strain may combine to produce a reassortant virus.

The National Institute of Allergy and Infectious Diseases (NIAID) has a long history of supporting rotavirus candidate vaccine development during the 1970s and 1980s, leading eventually to formation of a rhesus rotavirus quadrivalent vaccine expressing the most common human rotavirus serotypes: G1, G2, and G4, along with a rhesus G3. This vaccine advanced into clinical trials and was found to be safe and welltolerated. Upon its licensure in 1998 as RotaShield, it became the first rotavirus vaccine licensed in the United States. RotaShield was later voluntarily withdrawn from the market when data collected through postlicensure surveillance suggested an increase in a rare associated adverse event called intussusception. Currently, there are two licensed rotavirus vaccines.

RotaTeq (RV5) was initially developed by NIAID grantees and was licensed in the United States in 2006. It is a live oral human-bovine pentavalent reassortant rotavirus vaccine. RotaTeq is given to infants in three doses as an oral liquid at 2, 4, and 6 months. Large clinical trials showed no increase in intussusception with RotaTeq when compared to the placebo group. A threefold increase in serum immunoglobulin A (IgA) antibodies was seen in a subgroup of infants receiving RotaTeq, compared with those receiving placebo [6]. Efficacy against any rotavirus gastroenteritis matching the vaccine serotypes in the first year was 74 percent and rose to 98 percent against any severe rotavirus gastroenteritis.

Rotarix (RV1) is a live attenuated oral human vaccine containing only the most common human genotype, G1, yet proved in trials to protect against severe diarrhea for G1, G2, G3, G4, and G9 rotavirus strains. Given to infants in two doses between 6 and 24 weeks old, Rotarix was originally approved for use in more than 90 countries; it was licensed for use in the United States in 2008. No increase in intussusception was seen during clinical trials when comparing Rotarix to placebo.

Looking Forward

Current rotavirus vaccines have improved the health of children around the world. However, new vaccines could continue to reduce the global impact of rotaviruses. Together Rotarix and RotaTeq are licensed in more than 100 countries but remain cost-prohibitive for many developing countries. Considerations for next-generation vaccines include: affordability, ease of delivery, ambient storage, and use in higher-risk populations, such as infants with compromised immune systems or poor nutrition.

Isolates of human rotaviruses taken from asymptomatic infants are still considered a promising source of new vaccines. An example of government and private sector collaboration exists in the development of a vaccine that is now taking place in India. A naturally occurring rotavirus strain was isolated in a neonatal unit in India, adapted to Primary African Green Monkey Kidney (PAGMK) cells by CDC, and later transferred to NIAID for production of clinical lots. The resulting vaccine was tested in the United States by NIAID in adults and children. The vaccine was then transferred to a biotechnology company in India where it was adapted to Vero cells and tested in Phase I and II studies. The newly formulated vaccine is currently in Phase III studies in India under support from the Bill & Melinda Gates Foundation.

Other rotavirus vaccine candidates moving forward in clinical trials include an oral vaccine based on a neonatal strain of rotavirus and vaccines made from recombinant virus-like particles that are incapable of replication yet have proven effective against animal rotavirus. Another option being advanced uses killed rotavirus strains delivered by injection, in hopes that such vaccines may be more protective in higher risk populations, where oral vaccines are typically less effective.

In order to increase accessibility to rotavirus vaccines, NIAID has negotiated agreements with pharmaceutical companies in Brazil, China, and India for the transfer of human-bovine rotavirus vaccine technology and biological starting materials that have been developed by NIAID scientists. The goal is to have local companies make affordable vaccine, raising the hope that the vaccine will be incorporated into local programs and the disease burden will be reduced. The success of current rotavirus programs demonstrates that research on new prevention strategies, including vaccines, can make a significant impact on improving health and decreasing costs [7].

REFERENCES

1. World Health Organization. Immunization, vaccines and biologicals: rotavirus [Internet]. Geneva: World Health Organization; 2010 Apr 12. Accessed from: www.who.int/immunization/topics/rotavirus/en/index.html
2. Glass RI, Kilgore PE, Holman RC, Jin S, Smith JC, Woods PA, et al. The epidemiology of rotavirus diarrhea in the United States: surveillance and estimates of disease burden. J Infect Dis. 1996 Sep;174 Suppl 1:S5-11.
3. Widdowson MA, Meltzer MI, Zhang X, Bresee JS, Parashar UD, Glass RI. Cost effectiveness and potential impact of rotavirus vaccination in the United States. Pediatrics. 2007;119:684-97.
4. Patel MM, Steele D, Gentsch JR, Wecker J, Glass RI, Parashar U. Real-world impact of rotavirus vaccination. Pediatr Infect Dis J. 2011 Jan;30(1 Suppl):S1-5.
5. Cortes JE, Curns AT, Tate JE, Cortese MM, Patel MM, Zhou F, et al. Rotavirus vaccine and health care utilization for diarrhea in U.S. children. N Engl J Med. 2011 Sep 22;365:1108-17.
6. Block SL, Vesikari T, Goveia MG, Rivers SB, Adeyi BA, Dallas MJ, et al. Efficacy, immunogenicity, and safety of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine at the end of shelf life. Pediatrics. 2007 Jan;119:11-8.
7. Glass RI, Patel M, Parashar U. Lessons from the US rotavirus vaccination program. JAMA. 2011 Oct 19;306(15):1701-2.