

Selected Science Advances, 2009

The National Institute of Allergy and Infectious Diseases (NIAID) conducts and supports basic and applied research to better understand, treat, and ultimately prevent infectious, immunologic, and allergic diseases. In the past year, NIAID has conducted and supported many basic and clinical research studies that have improved our understanding of disease and advanced the quest for new treatments and prevention strategies. Some of the most exciting discoveries are highlighted below.

Rapid characterization of the 2009 H1N1 influenza virus

In April 2009, the 2009 H1N1 influenza virus emerged in Mexico and the United States. It quickly spread worldwide to cause the first influenza pandemic of the 21st century. Just 2 months after its emergence, the virus genome was decoded by investigators from the NIAID Centers of Excellence for Influenza Research and Surveillance. They found that the virus had genomic pieces from avian, human, and swine flu viruses. Within 4 months, the researchers had analyzed several other properties of the new influenza virus as well. Using a ferret model, they made several key observations when they compared the 2009 virus with seasonal H1N1 flu viruses: The 2009 H1N1 virus was more pathogenic, it infected cells deeper in the lungs, it replicated more efficiently, and in terms of establishing infection, it dominated the other H1N1 viruses. The researchers also found that the 2009 H1N1 influenza virus is sensitive to some approved and experimental antiviral drugs. Finally, analyses of human blood samples taken from individuals in different age groups indicated that many older individuals had some natural immunity to both 2009 H1N1 and 1918 pandemic influenza viruses, indicating the genetic relatedness between the two viruses.

References: G Smith *et al. Nature* 459:1122-25 (2009); V Munster *et al. Science* 325:481-83 (2009); Y Itoh *et al. Nature* 460:1021-25 (2009); D Perez *et al. PLoS Curr Influenza* 24:RRN1011 (2009).

Drug shows promise against pandemic flu strains

To combat novel as well as drug-resistant influenza strains, additional antiviral approaches are needed. The drug Fludase (DAS181), a synthetic protein developed to prevent entry of flu viruses into respiratory cells, has shown activity against various human seasonal flu viruses as well as potentially lethal avian flu strains. Investigators recently studied the effectiveness of Fludase against several pandemic H1N1 influenza

viruses, including the 1918 pandemic strain. They found that the drug stopped replication of the pandemic flu strains in cell culture models and protected mice from pandemic flu disease. Its antiviral activity was similar against pandemic influenza strains and seasonal flu virus, including seasonal flu strains that are resistant to oseltamivir (Tamiflu). These results suggest that Fludase may provide protection should pandemic influenza viruses, such as 2009 H1N1, develop widespread resistance to Tamiflu. NIAID currently is supporting clinical trials to study the effectiveness of Fludase in treating people with noncomplicated influenza infection.

References: G Triana-Baltzer *et al. PLoS One* 4:e7788 (2009) and 4:e7838 (2009).

Flu's Achilles' heel: Scientists identify proteins that neutralize multiple strains of seasonal and pandemic flu viruses

Two independent research teams supported by NIAID have discovered a common Achilles' heel in a wide range of seasonal and pandemic influenza A viruses. The pair of studies found a small family of infection-fighting proteins, or human antibodies, that neutralize various influenza A virus subtypes by attaching to these viruses in the same place. This common attachment site provides a constant region of the flu virus for scientists to target in an effort to develop a so-called universal flu vaccine. Such a vaccine would overcome the annual struggle to make the seasonal flu vaccine match next year's circulating flu strains and might help curtail the transmission of emerging pandemic influenza viruses as well. Lab-made human monoclonal antibodies based on the neutralizing antibodies identified also might be made relatively quickly and potentially could be used in combination with antiviral drugs to prevent or treat the flu during an influenza outbreak or pandemic.

References: J Sui *et al. Nat Struct Mol Biol* 16:265-73 (2009); D Ekiert *et al. Science* 324:246-51 (2009).



A first: HIV vaccine shows modest preventive effect

For the first time in more than two decades of research, an experimental HIV vaccine has demonstrated a modest preventive effect against HIV infection among vaccinated individuals enrolled in a major clinical study. The study, headed by the U.S. Army Surgeon General and largely funded by NIAID, involved more than 16,000 men and women in Thailand. The trial tested the safety and effectiveness of a prime-boost regimen of two vaccines: ALVAC-HIV vaccine (the primer dose), a modified canarypox vaccine; and AIDSVAX B/E vaccine (the booster dose), a recombinant vaccine based on the HIV surface protein gp120. In the final analysis, the vaccine regimen was found to be 31 percent effective at preventing HIV infection. Specifically, 74 of 8,198 placebo recipients became infected with HIV compared with 51 of 8,197 participants who received the vaccine regimen. In light of the findings, NIAID and other scientific leaders are working together to explore what the Thai study results mean for future HIV vaccine research.

Reference: S Rerks-Ngarm et al. New Engl J Med 361:2209-20 (2009).

Starting anti-HIV therapy earlier yields better clinical outcomes

NIAID-funded research contributed to the World Health Organization's (WHO's) recent decision to change its guidelines for starting antiretroviral therapy (ART) earlier in HIV-infected persons in resource-limited countries. The guidelines now recommend starting treatment when a person's CD4+ T cell blood count—a measure of immune health—falls below 350 cells per cubic microliter. Until recently, in many countries it had been standard practice to start ART when the CD4+ count dropped below 200. For comparison, a healthy individual typically has a CD4 count of between 750 and 1,500. Findings from the NIAID-funded research added to the momentum for change in WHO guidelines. In one instance, scientists modeled HIV disease using data from published studies of HIV-infected individuals in South Africa. Their model found that if ART were begun at a CD4 count of 350, severe opportunistic disease and deaths over the next 5 years could be reduced dramatically. This strategy would increase long-term survival by more than 8 years and could lead to significant cost savings. In the second instance, an independent monitoring board conducted an interim review of a clinical study of HIV-infected adults in Haiti. The board found overwhelming evidence to support what the other investigators have shown: Starting ART at CD4 counts of 200 to 350 improved survival, compared with deferring treatment. With these findings, the board recommended that

NIAID end the trial immediately and offer ART to all participants with CD4 counts less than 350. In light of accumulating evidence, at the end of 2009, WHO changed the standard of care for treatment of HIV infection in dozens of countries around the world.

Reference: R Walensky et al. Ann Int Med 151:157-66 (2009).

New HIV vaccine target revealed by studies of neutralizing antibodies

Researchers have developed two new methods to quickly generate neutralizing antibodies against several variants of HIV found outside North America and Europe. This advance should assist in the design of new HIV vaccines that elicit antibodies that protect against a broad array of HIV subtypes. In both methods, the source antibodies were derived from the blood of HIV-infected individuals. The scientists first assessed the neutralizing potential of blood samples taken from more than 1,800 HIV-infected individuals from Thailand, Australia, the United Kingdom, the United States, and several sub-Saharan African countries. Those samples that exhibited broad, strong neutralizing activity against subtypes of HIV found outside North America and Europe were selected as sources for generating monoclonal antibodies, or identical clones of the original antibodies. Then, the scientists used an efficient technique to screen thousands of antibody-containing samples from an African donor. From those, they isolated two potent antibodies with broadly neutralizing potential. These isolated antibodies are particularly important because they target a stable region of the HIV surface protein gp120, which otherwise has a high rate of viral mutation that allows HIV to escape traditional immune defenses.

Reference: L Walker et al. Science 326:285-89 (2009).

Clue to HIV structure may help scientists design better HIV vaccines

The site on the HIV surface protein gp120 that binds to the CD4 receptor on T cells is vulnerable to antibodies. The vast majority of antibodies that recognize this site, however, do not prevent HIV infection. Researchers at the NIAID Vaccine Research Center recently discovered the structural basis for this immune evasion. These scientists showed, in atomic detail, how the conformational flexibility of gp120 facilitates a decoy strategy that misdirects the antibody response. These results would explain why even slight variations in the angle of antibody approach to the functional spike—the cluster of three gp120 molecules that enables the virus to infect T cells—prevent most antibodies from neutralizing the virus. The findings suggest structural-based strategies

that could be used to design novel ways to generate HIV-neutralizing antibodies. New discoveries about anti-HIV antibodies may bring researchers a step closer to creating an effective HIV vaccine.

Reference: L Chen *et al. Science* 326:1123-27 (2009).

Pre-exposure prophylaxis as HIV prevention could yield substantial benefits, mathematical model finds

Providing antiretroviral drugs to people who are not infected with HIV but who are at high risk of infection—an investigational approach known as pre-exposure prophylaxis (PrEP)—could substantially reduce their lifetime risk of HIV infection and could be as cost-effective as other widely recommended public health and medical interventions, according to a mathematical model developed by NIAID grantees at Yale University. As a basis for their model, the researchers assumed 1) PrEP is 50 percent effective; 2) the target population is American men who have sex with men who are on average 34 years of age; 3) 1.6 percent of this population becomes newly infected with HIV annually; and 4) the antiretroviral drugs (tenofovir and emtricitabine) cost \$9,000 annually. Within these parameters, the model predicts PrEP would cut the lifetime risk of HIV infection from 44 percent to 25 percent. The model predicted that PrEP in this hypothetical setting would not be cost effective under current U.S. standards. However, the researchers assert that by changing the model’s assumptions, for instance incrementally increasing the protective effect of PrEP, decreasing the drug costs, or targeting a higher risk population, PrEP could be a cost-effective approach to HIV prevention.

Reference: A Paltiel *et al. Clin Infect Dis* 486:806-15 (2009).

Memory B cells provide insight into HIV-specific neutralizing antibodies

Many researchers believe that a vaccine to prevent HIV infection must stimulate the body to make neutralizing antibodies, infection-fighting proteins that in the case of HIV prevent the virus from infecting immune cells. Using state-of-the-art technology, researchers at NIAID’s Vaccine Research Center for the first time are now examining how neutralizing antibodies develop during natural HIV infection, which could provide key clues to developing an effective HIV vaccine. By collecting antibody-producing memory B cells from HIV-infected individuals and incubating the cells with a specially flagged protein from the outer shell of an HIV virus particle, the HIV-specific cells bind to the protein, enabling researchers to identify the cells and isolate and store them. For each of the HIV-specific

memory B cells, a pioneering technique is then used to express the genes that code for HIV-specific antibodies, and assays are used to help scientists determine which of the antibodies can effectively neutralize HIV.

Reference: J Scheid *et al. Nature* 458:636-40 (2009).

Male circumcision helps prevent HSV-2 and HPV infection

Medical circumcision can help heterosexual men significantly reduce their risk of acquiring two common sexually transmitted infections—herpes simplex virus type 2 (HSV-2), the cause of genital herpes, and human papillomavirus (HPV), which can cause cancer and genital warts. The findings build upon earlier NIAID-funded clinical research, which found that circumcision decreases a man’s risk of acquiring HIV infection through heterosexual intercourse by more than 60 percent. The new study is based on two clinical trials at sites in Uganda involving 3,393 uncircumcised men between the ages of 15 and 49. Overall, researchers found that circumcision reduced the men’s risk of HSV-2 infection by 28 percent and reduced HPV prevalence by 35 percent. Circumcision did not, however, affect the incidence of syphilis.

Reference: A Tobian *et al. New Engl J Med* 360:1298-1309 (2009).

Experimental cytomegalovirus vaccine shows promise

Every year, approximately 8,000 infants in the United States develop severe hearing, mental, or motor impairments after becoming infected with cytomegalovirus (CMV) while still in the womb. A vaccine to prevent congenital CMV infection has long been a public health priority, but has proved elusive. An NIAID-supported clinical trial enrolled 441 CMV-negative women to receive either an experimental CMV vaccine or a placebo. Women who received the vaccine were half as likely as woman who did not to later become infected with CMV. While larger studies are needed to confirm the possible effectiveness of this or any other CMV vaccine at preventing congenital CMV infection, the results do give rise to optimism that such a vaccine may be closer.

Reference: R Pass *et al. New Engl J Med* 360:1250-52 (2009).

Immune therapy helps peanut-allergic people eat peanuts

Approximately 1 percent of people in the United States are allergic to peanuts, many of whom are children who will not outgrow their allergy. Peanuts induce most life-threatening allergic reactions to food and are the most common cause of food allergy-related death. Currently, the best way to manage peanut allergy is to avoid peanuts and peanut-containing foods. Scientists supported by NIAID are exploring oral immunotherapy to treat peanut allergy. In oral immunotherapy, a person consumes gradually increasing amounts of the food allergen to build immune tolerance to the food. The goal is to develop long-lasting tolerance so that the person can eat the food without having an allergic reaction. Recently, investigators treated 29 people who have peanut allergy with oral immunotherapy. After 8 months, 27 of the 29 individuals were able to eat the equivalent of approximately 16 peanuts without triggering an allergic response. In addition, their peanut-specific immunoglobulin (Ig) E antibodies had declined and their concentration of white blood cells that dampen the immune response had increased. This study suggests that oral immunotherapy may help people who have peanut allergy tolerate increasingly higher doses of peanuts.

Reference: S Jones *et al.* *J Allergy Clin Immunol* 124:292-300 (2009).

Scientists identify biomarkers of kidney transplant tolerance

NIAID-supported investigators have found that microRNAs (miRNAs), which are small pieces of nucleic acids that regulate gene expression, can determine the health and function of transplanted kidneys. In this study, investigators looked at expression patterns of several miRNAs in biopsies from kidney transplant patients. Patients undergoing acute rejection had different patterns of expression compared with those seen in recipients who have normal functioning transplants. In addition, when they compared the miRNA levels in the kidney biopsies to those measured in white blood cells of the same patients, the patterns were similar. These results suggest that measuring miRNA levels in a kidney recipient's blood may be useful for diagnosing rejection and predicting how well a transplant is functioning—thereby avoiding the need for a kidney biopsy—and also may be useful for tailoring medications to the needs of individual patients.

Reference: D Anglicheau. *Proc Natl Acad Sci USA* 106:5330-35 (2009).

Possible new primary immune deficiency disease identified

Primary immune deficiency diseases (PIDDs) are caused by inherited defects in specific cells of the immune system. People who have PIDDs generally have an increased susceptibility to infections and may have other medical problems that include autoimmune diseases, weakened lung function, tumors, and failure to thrive. NIH investigators have found a genetic mutation that accounts for a previously unidentified immune disease in 11 patients receiving treatment in the NIAID Primary Immune Deficiency Clinic. While the patients received care for their symptoms—including persistent skin infections, acute allergies, and cancer—investigators observed that they all had a mutation in the same gene, DOCK8. The normal function of DOCK8 is unknown. But the people who had DOCK8 mutations had fewer anti-virus immune cells, fewer antibody-producing cells, and increased numbers of white blood cells associated with allergy, suggesting that DOCK8 is essential for protection against viruses and prevention of the development of allergies and cancers. Although more study is needed to determine whether DOCK8 mutations occur in other people who have similar symptoms, DOCK8 immunodeficiency syndrome may be a new PIDD. Identifying a cause for the disease has provided comfort to some of those diagnosed who had battled an unknown immune disease for years.

Reference: Q Zhang *et al.* *N Engl J Med* 361:2046-55 (2009).

Nonimmune cells contribute to the immune response to airborne allergens

In a mouse study, NIAID-supported scientists discovered that when special sensors called Toll-like receptors (TLRs)—which dot the surface of epithelial cells that line the lungs—detect the presence of airborne allergens, the sensors activate immune cells. The researchers observed that a particular TLR, TLR4, was sensitive to bacteria and dust mites. Previously, it was unclear whether TLRs on nonimmune epithelial cells at mucosal surfaces such as those in the lungs were involved in antigen sensing, or whether it was TLRs found on immune cells in these areas that were critical to these allergic responses. The research team observed that TLR4 on airway epithelial cells, not on immune cells, helped induce the initial immune response to allergens in the lungs. The new results suggest that targeting TLRs may be a research avenue for developing novel treatments for allergic diseases such as asthma.

Reference: H Hammad *et al.* *Nat Med* 15:410-16 (2009).

Adaptive features observed in innate immune cells

Natural killer (NK) cells are a type of white blood cell traditionally thought to be part of the innate, or inborn, immune system. Cells of the innate immune system provide a rapid, nonspecific response when exposed to a microbe for the first time. They do not expand or contract in number after exposure to a microbe, and behave as if they had never seen the microbe the next time they are exposed. This is in contrast to cells of the adaptive immune system that increase and decrease in number during an infection and, having seen the microbe once, retain a memory of the specific infection and are able to respond more efficiently the second time around. Recently, two groups of NIAID-supported investigators showed that NK cells may have features of adaptive immune cells.

The first team observed that NK cells from mice exposed to a virus increased in number during the response to the initial infection and then contracted in number after the infection was cleared. Some virus-specific “memory” NK cells remained in circulation, and their number increased more rapidly after a second infection. The second team showed that activating NK cells with signaling molecules, called cytokines, led to stronger NK responses upon each subsequent exposure. Taken together, these two studies indicate that NK cells have never-before realized features of adaptive immune cells and thus could be considered novel targets for future vaccine development.

References: J Sun *et al.* *Nature* 457:557-61 (2009); M Cooper *et al.* *Proc Natl Acad Sci* 106:1915-19 (2009).



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