

Selected Science Advances, 2010

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. During fiscal year 2010, NIAID conducted and supported many basic and clinical research studies that improved our understanding of disease and advanced the quest for new treatments and prevention strategies. Some of the most exciting discoveries are highlighted below.

Daily dose of HIV drug reduces risk of infection

In a major advance for HIV prevention, NIAID-supported researchers found that a daily dose of an oral antiretroviral drug, currently approved to treat HIV infection, reduced the risk of HIV infection among men who have sex with men (MSM) by 43.8 percent. The drug was even more effective (72.8 percent) among those who closely adhered to the drug regimen.

The study, known as iPrEx, involved 2,499 adult MSM and transgendered women who have sex with men at 11 sites in Brazil, Ecuador, Peru, South Africa, Thailand, and the United States. The participants were randomly assigned to receive either a daily antiretroviral pill containing combination emtricitabine and tenofovir, known by the brand name Truvada, or a placebo tablet. In the final analysis, 100 cases of HIV infection occurred among all of the iPrEx study participants. Of those, 36 HIV infections occurred among those who received Truvada, compared with 64 infections among the placebo group. The drug appeared to be safe and well-tolerated with mild and infrequent side effects reported among a small number of participants. Additionally, very little drug resistance occurred, with no instances of tenofovir resistance and three cases of emtricitabine resistance. Further, both groups of study participants reported a decrease in the number of sexual partners and increased condom use.

The iPrEx results pertain only to MSM. Other studies are evaluating the HIV prevention strategy among women, injection drug users, and heterosexual couples where one partner is infected with HIV and the other is not.

Reference: RM Grant *et al.* *N Engl J Med.* 363(27):2587-99 (2010).

Earlier start to HIV treatment reduces risk of sickness, death

The optimal time for HIV-infected asymptomatic people to begin antiretroviral treatment remains to be established.

In Haiti, the standard of care is to provide antiretroviral treatment when an HIV-infected patient's CD4+ T-cell count, a key measure of immune system health, drops below 200 cells per cubic millimeter (mm³). An NIAID-funded study sought to determine whether HIV-infected patients in Haiti would experience better health outcomes in comparison to the standard of care.

Of the 816 HIV-infected participants in the Haiti study, half were randomly assigned to receive the anti-HIV drugs zidovudine, lamivudine, and efavirenz within 2 weeks of enrollment. The remaining half began the trio of antiretrovirals when CD4 counts dropped below 200 cells/mm³. The average CD4 count in both groups at time of enrollment was 280 cells/mm³. Twenty-three deaths occurred among those who received the standard of care, compared with six deaths among those in the early-treatment group. Twice as many participants in the standard-of-care group developed tuberculosis infection.

Based on the findings, researchers recommended that international HIV treatment guidelines be revised to provide antiretroviral access to HIV-infected patients with CD4 counts of less than 350 cells/mm³. To date, only 30 percent of HIV-infected individuals in low- and middle-income countries who need antiretrovirals have access to the life-saving medications.

Reference: P Severe *et al.* *N Engl J Med.* 363(3):257-65 (2010).



Possible new treatment for severe disease of the blood vessels

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis is a rare but devastating autoimmune disease affecting the blood vessels. In this disease, antibodies attack immune cells called neutrophils, causing inflammation in small- to medium-sized blood vessels. This leads to organ damage, particularly in the airways, lungs, and kidneys, and to recurring symptoms such as fever, fatigue, and bleeding in the lungs. Treatment usually consists of a 3- to 6-month course of cyclophosphamide—which suppresses immune cells known as B cells that produce the self-destructive antibodies—followed by long-term daily use of other immune-suppressing drugs plus steroids. Before the cyclophosphamide-based treatment was developed by NIAID investigators in the 1970s, about 80 percent of people with ANCA-associated vasculitis died within 2 years of disease onset. While the drug has been a lifesaver, its long-term use puts patients at increased risk of infection, cancer, and infertility. In a recent study, NIAID-supported researchers evaluated a new treatment strategy based on the drug rituximab, a synthetic antibody that selectively reduces the number of circulating B cells. The researchers found that rituximab provided the same benefits as cyclophosphamide but required a shorter course of treatment. Early results also suggest that people with relapsing disease respond better to rituximab than cyclophosphamide: In the trial, 67 percent of those individuals given rituximab were able to stop all steroid use, compared with only 42 percent of those given standard care.

Reference: JH Stone *et al.* *N Engl J Med.* 363(3):221-32 (2010).

Scientists find new prion disease that damages brain arteries

Creutzfeldt-Jakob disease in humans and scrapie in sheep are examples of transmissible spongiform encephalopathies (TSEs), fatal diseases that kill brain cells. Scientists believe TSEs are caused by the buildup of abnormal forms of prion protein that causes sponge-like holes in the brain. Using special mice, NIAID researchers have found a new prion disease that does not make holes in the brain. Instead, the disease damages brain blood vessels in a process called cerebral amyloid angiopathy, an outcome typical of people with Alzheimer's disease. The study mice first had a molecular anchor genetically removed to prevent prion protein from attaching to brain cells. Researchers then infected the mice with scrapie and observed them for 500 days. Upon examination, the mouse brains showed large

accumulations of prion proteins trapped outside blood vessels, and no holes in the brain. Mouse symptoms resembled the neurologic symptoms seen in people with cerebral amyloid angiopathy, which increases the risk of profuse bleeding and dementia. This is the first animal model to show that abnormal prion protein accumulation can damage blood vessels without making holes in the brain. If scientists learn how to stop this new prion disease in the mouse, they might apply the same method to treat people with Alzheimer's disease.

Reference: B Chesebro *et al.* *PLoS Pathogens.* 6(3):e1000800. doi.101371/journal.ppat.1000800 (2010).

Gene therapy improves health of people with inherited immune disorder

Chronic granulomatous disease (CGD) is a rare but serious inherited disorder of the immune system. People with CGD have an increased susceptibility to bacterial and fungal infections and to sores in the lungs, liver, spleen, bones, or skin. Those with severe disease also can develop tissue masses, called granulomas, which can obstruct the bowel or urinary tract. CGD is caused by inherited mutations in any one of five different genes. Immune cells called neutrophils require these genes to make an enzyme that helps destroy harmful bacteria and fungi. Most people with CGD inherit the mutation as an X-linked trait, meaning that the abnormal gene sits on the female sex chromosome. X-linked CGD (X-CGD) results from mutations of the gene *CYBB*, which makes gp91. The protein gp91 is part of the infection-fighting enzyme. In this study, NIAID-supported investigators gave three patients with severe X-CGD a form of gene therapy designed to produce a normal version of the gp91 protein. The patients also received busulfan, a chemotherapy drug that improves the clinical benefits of gene therapy. Two of the patients treated with gene therapy plus busulfan were able to make neutrophils that produced the functional enzyme and sufficient levels of microbe-killing molecules to at least partially control their infections.

Reference: EM Kang *et al.* *Blood.* 115(4):783-91 (2010).

Anti-HIV therapy early in infection may preserve immune function

HIV-infected individuals who begin antiretroviral therapy (ART) soon after infection may have stronger immune responses to other disease-causing organisms than HIV-infected individuals who begin ART later in the course of HIV disease, according to recent research. NIAID scientists measured the quantity and qualities of B cells in blood samples taken from three groups

of volunteers: men infected with HIV for fewer than 6 months; men infected with HIV for 6 months or more; and men who were not infected with HIV. B cells make proteins called antibodies that can flag pathogens for destruction and prevent them from infecting cells. Once the two groups of HIV-infected men began ART, the number of B cells in their blood increased significantly and to similar degrees, but the composition of B cells in each group differed notably throughout the study. To learn how these differences affected immune responses to new infections, the researchers examined how the HIV-infected men responded to influenza vaccination at study initiation and 1 year after beginning treatment. At the 1-year point, a significantly greater proportion of B cells made anti-influenza antibodies in the early treatment group, compared with the late treatment group. This suggests that starting ART earlier may prevent irreversible immune system damage and adds to the body of evidence showing significant health benefits from early ART.

Reference: S Moir *et al.* *Blood*. 116:5571-9 (2010).

Diagnostic provides rapid detection of TB and its drug-resistant form

Current tests to diagnose tuberculosis (TB) and determine whether the bacterium is of a drug-resistant variety can take weeks, leaving affected patients without treatment and potentially exposing the broader population to infection. Through an NIAID-funded public-private collaboration, researchers developed a test that can detect TB and resistance to rifampin, an antibiotic commonly used to treat TB, within 2 hours. The molecular test, known as the Xpert MTb assay, uses a patient's sputum to identify the presence of *Mycobacterium tuberculosis* (MTb), the bacterium that causes TB, and rifampin resistance without the need for lengthy bacteria cultures to prove a diagnosis. The assay, which is included in a commercially available diagnostic, is as sensitive as currently available methods, but has the added benefits of allowing for easier and safer disposal and controls to prevent operational errors.

Simple to use, the test enables patients to receive their diagnosis during a single clinic visit. As part of a larger system, the assay also may provide a cost-effective approach for the diagnosis of other infectious diseases. The World Health Organization recently endorsed the Xpert MTb assay for further evaluation and use in the global diagnosis of drug-resistant TB.

Reference: R Blakemore *et al.* *J Clin Microbiol*. 48(7):2495-501 (2010).

Stalking a universal flu vaccine

Ever-changing influenza virus strains require that new vaccines be developed each flu season—a costly and time-consuming task. The most changeable part of a key influenza protein, called hemagglutinin (HA), is its globular “head” region, which current influenza vaccines target. In contrast, HA's stalk varies relatively little from strain to strain. In two related studies, NIAID-funded scientists made a vaccine using only the stalk of HA and used it to immunize mice. When later exposed to whole flu viruses, mice receiving the stalk-only vaccine mounted a broader immune response than mice that received a vaccine made from whole HA protein. The stalk-only vaccine also provided one group of mice with partial protection from infection, and another group of vaccinated mice had complete protection from otherwise lethal levels of flu virus. Together, the studies represent a foundation for further development of an influenza vaccine based on HA's stalk that could confer protection against many strains of flu. Such a broadly protective, “universal” flu vaccine has the potential to last for multiple years and guard against many or most flu strains—a clear advantage over today's flu vaccines.

References: J Steel *et al.* Influenza virus vaccine based on the conserved hemagglutinin stalk domain. *MBio*. 1(1):e00018-10 (2010); TT Wang *et al.* Vaccination with a synthetic peptide from the influenza virus hemagglutinin provides protection against distinct viral subtypes. *PNAS*. 107(44):18979-84 (2010).

Drug shows promise against pandemic flu strains

To combat new and 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 how well Fludase does 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; it protected mice from pandemic flu disease; and its antiviral activity against pandemic influenza strains was about the same as that observed against seasonal flu virus, including oseltamivir (Tamiflu)-resistant viruses. These results suggest that Fludase may provide protection should pandemic viruses, such as H1N1, develop resistance to Tamiflu. NIAID is currently testing the drug in people with influenza infection.

Reference: B Gallen *et al.* *PLoS One*. 4:e7788 (2009).

New strategy provides evidence for future universal flu vaccine

The emergence and rapid spread of the H1N1 influenza virus in 2009 made clear the need for a “universal” flu vaccine—one that could protect for decades against multiple, if not all, flu strains. In 2010, NIAID scientists took a step toward making this a reality with a two-step immunization approach designed to elicit infection-fighting antibodies that attacked a range of flu virus strains when tested in mice, ferrets, and monkeys. The researchers first primed, or activated, the animal immune systems with a vaccine made from DNA encoding the flu virus’ hemagglutinin (HA) surface protein. The animals then received a booster dose of the 2006–2007 seasonal influenza vaccine.

The researchers found that although the DNA in the priming vaccine was derived from a 1999 flu virus, the animals produced antibodies capable of neutralizing a wide range of flu virus strains, including those from 1934, 2006, and 2007. Additionally, although the prime-boost vaccines were made from H1 subtypes of influenza A, they also neutralized other types of flu, including H5N1 (avian influenza), suggesting that a prime-boost strategy could protect against many or all subtypes of influenza A. Further analysis of the neutralizing antibodies revealed that they targeted a portion of the HA protein that varies little from strain to strain, giving hope that a vaccine strategy could confer immunity against multiple flu strains. Clinical trials are currently underway in humans to test the safety and efficacy of prime-boost influenza vaccines.

Reference: Wei CJ, Boyington JC, McTamney PM, *et al.* *Science*. 329(5995):1060-4 (2010).

New antibodies block infection by vast majority of HIV strains

Scientists led by a team from the NIAID Vaccine Research Center (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. The researchers also demonstrated how one of these infection-preventing proteins accomplishes this feat. These advances have enabled the scientists to design a candidate vaccine that could elicit antibodies similar to the ones they discovered. Such a vaccine may prevent infection by the vast majority of HIV strains worldwide. The scientists found the powerful new antibodies, named VRC01 and VRC02, using a protein they developed that reacts only with antibodies that bind to the area HIV uses to attach to and infect immune cells. This area, called the CD4 binding site, is one of the few parts of the viral surface that remains

nearly constant across the globe. By attaching to the CD4 binding site, VRC01 and VRC02 block HIV infection by more viral strains with greater overall strength than any previously known antibodies. The researchers examined the atomic-level protein structure of VRC01, enabling them to define how VRC01 works and locate precisely where it attaches to HIV. The new antibodies could be used in the design of not only HIV vaccines, but also microbicides and therapeutics.

References: T Zhou *et al.* *Science*. 329:811-7 (2010); X Wu *et al.* *Science*. 329:856-61 (2010).



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