CHIPPING AWAY AT HIV
NEW STRATEGIES EMERGE FOR VANQUISHING THE VIRUS
Measles is back. After declaring it eliminated in 2000, the United States is now dealing with an uptick in cases. The latest outbreak began in California’s Disneyland theme park last December and, by early April, had ballooned to 159 cases across 18 states, according to the US Centers for Disease Control and Prevention (CDC). This troubling situation serves as a stark reminder of the importance of immunization, which for many years had kept this once ubiquitous and sometimes deadly childhood disease in check. Developed in the late 1960s under the guidance of longtime Merck scientist Maurice Hilleman, the two-dose regimen of the combination vaccine against measles, mumps, and rubella (MMR) is estimated to be 97 percent effective at preventing measles in a vaccinated individual.

But developing vaccines against measles and other diseases was relatively straightforward when compared with the process scientists must use today to fight much more complex pathogens, such as HIV. Part of what makes HIV such a difficult virus to combat is its ability to furiously mutate the trimeric envelope protein on its outer surface. This high mutation rate gives rise to multiple HIV subtypes that circulate around the globe, allowing the virus to escape the responses that human immune systems mount against it. The approach that proved so successful for the MMR vaccine—using attenuated versions of the pathogens to immunize people—isn’t feasible for HIV because of concerns that the virus could mutate and regain its pathogenicity. And using a killed or inactivated virus—the other classic approach of vaccinology, used

Recent discoveries are spurring a renaissance in HIV vaccine research and development.

BY WAYNE C. KOFF
to develop vaccines against polio and influenza viruses, among others—doesn’t effectively address the unprecedented genetic variability of HIV.

There are other challenges unique to HIV. Quickly following transmission, the virus disseminates and establishes a persistent infection, including hidden reservoirs from which it can strike again at any time. (See “Hidden Menace” on page 34.) The opportunity for a vaccine-induced response to prevent infection or to control the initial, limited infection is thus short-lived. And while many people mount an effective immune response to and recover from most other viral infections, not a single person infected with HIV has cleared the virus on his or her own. The lone individual considered cured of HIV—Timothy Ray Brown, also known as the Berlin patient—only reached this milestone after receiving two bone marrow transplants to treat acute myeloid leukemia, which he’d developed after a decade of living with HIV and taking antiretroviral drugs. Doctors deliberately chose a stem cell donor with a genetic mutation that is known to confer resistance to HIV infection, in addition to a panoply of other chemotherapies and immune suppressive treatments to treat his acute myeloid leukemia. Attempts to repeat the success of this complex approach in other individuals with both cancer and HIV have so far been futile.

Scientists still don’t understand how to elicit specific, durable, and protective immune responses against HIV. Animal models, while informative, can only hint at what works. This means HIV vaccine researchers need to be as wily as the virus we are trying to combat. Progress during the past five years is spurring creative and promising new approaches. Armed with intriguing results from clinical trials and tremendous progress in isolating and understanding the evolution of broadly neutralizing antibodies against HIV, the field is now poised to elucidate the rules of immunogenicity and accelerate progress toward an effective vaccine.

Success cannot come too soon. Despite considerable advances in preventing new HIV infections and in delivering lifesaving treatment to those already infected, 2.1 million people worldwide contracted HIV in 2013, according to the Joint United Nations Programme on HIV/AIDS (UNAIDS). In the same year, some 1.6 million people died of HIV/AIDS or related complications. Altogether, since it was identified in 1983, HIV has infected 78 million people and killed half of them. An effective vaccine is a critical component to ending the morbidity and mortality caused by the disease.

**Clues from trials**

The HIV vaccine field has had its fair share of disappointing results from large, late-stage clinical trials. In 2007, vaccinations were stopped in the STEP and Phambili trials of a vaccine candidate that used replication-defective adenovirus serotype 5 (Ad5) to deliver HIV antigens designed to induce cellular immune responses against HIV. Then in 2013, vaccinations were terminated in the HVTN 505 trial, which tested a different Ad5 candidate in a prime/boost combination with a DNA-based vaccine. All three candidates failed to prevent HIV infection or blunt the disease’s course in those who became infected.

But in 2009, the field did get a first, albeit modest, clinical signal for feasibility of an HIV vaccine in humans, when scientists at the US Military HIV Research Program (MHRP) reported that a prime/boost combination of two different vaccines reduced the rate of HIV infection by 31.2 percent in more than 16,000 volunteers in Thailand. That trial, known as RV144, tested the canary-pox virus–vectored vaccine candidate ALVAC-HIV, followed by a modified HIV gp120 protein subunit vaccine named AIDSVAX gp120 B/E, which had provided no protection in previous efficacy trials when administered on its own.

Researchers are working to determine the immune responses that led to this modest level of protection. Meanwhile, further insights may come from a new round of clinical efficacy trials for this prime/boost combination. Expected to begin in South Africa in late 2016, the new trials are designed to evaluate modifications to the vaccine candidates and regimen, including testing related HIV immunogens, different adjuvants, and new immunization schedules with additional booster shots intended to improve both strength and durability of immune responses.

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**Going broad**

Researchers widely agree that an ideal HIV vaccine would induce the production of so-called broadly neutralizing antibodies, which are capable of neutralizing a broad swath of HIV strains and are produced naturally by approximately 25 percent of chronically HIV-infected people. To accomplish this, researchers must first identify what immunogens can elicit such a response. Although this remains a challenge, some scientists are making significant progress by employing reverse-engineering or structure-assisted vaccine discovery. This new approach starts with isolating broadly neutralizing antibodies from chronically infected HIV patients whose immune systems produce them. Researchers can then identify an antibody’s target on the virus, use the molecular structure of this target site to design immunogens that mimic these sites, and immunize volunteers with these mimics to try to elicit the desired antibody response.

HIV vaccine researchers were buoyed recently by promising results from the use of this structure-based design strategy to produce a vaccine candidate against pediatric respiratory syncytial virus (RSV), which is the leading cause of hospitalization for...
children under five years of age worldwide. Peter Kwong and colleagues at the Vaccine Research Center (VRC) of the US National Institute of Allergy and Infectious Diseases first identified a site on an RSV envelope glycoprotein that extremely potent neutralizing antibodies target before the virus fuses with the host cell membrane. The researchers then identified and incorporated a series of mutations to stabilize the RSV protein in this conformation, engineered a version of the target site, and used it to immunize mice and rhesus macaques, eliciting high titers of neutralizing antibodies against RSV in both species.

Similar results were also observed after vaccination with computationally derived RSV proteins. With these proof-of-principle studies demonstrating the effectiveness of this approach, coupled with recent advances in identifying HIV-specific broadly neutralizing antibodies, HIV vaccine researchers are now working to apply these principles to design and screen new vaccine candidates. In 2009, a consortium of research institutions reported the isolation of two potent broadly neutralizing antibodies from an HIV-infected donor who was part of a large cohort study led by the International AIDS Vaccine Initiative (IAVI), where I serve as chief scientific officer. These new antibodies neutralized HIV at 10- to 100-fold lower concentrations than the previously identified antibodies and were effective against a broader swath of viruses. This finding kicked off a flurry of new antibody discoveries, leading to the isolation of hundreds of HIV-specific broadly neutralizing antibodies, many targeting a relatively small number of specific sites on the virus. Characterization of these target sites has led to identification of the molecular structures of at least four highly conserved regions on HIV's envelope protein that can now be used to design vaccine immunogens. (See illustration on page 44.)

This boon in antibody isolation and characterization represents a major advance for structure-based HIV vaccine design efforts. Encouragingly, these antibodies can protect monkeys from infection with a hybrid simian/human immunodeficiency virus (SHIV), suggesting that a vaccine capable of inducing them in humans may afford protection against HIV.

Another major advance toward developing an effective HIV vaccine came in 2013 when a team of researchers led by John Moore at Weill Cornell Medical College in New York City and Ian Wilson at the Scripps Research Institute in La Jolla, California, obtained an atomic-level image of the HIV envelope trimer, the principal target for broadly neutralizing antibodies. To capture this detailed image, the researchers first had to engineer a more stable form of this notoriously unstable protein, then use cryo-electron microscopy and X-ray crystallography to reveal its structure. A high-resolution structural model of the pre-fusion, closed form of HIV envelope by Kwong and colleagues at the VRC soon followed. The vaccine field had been stymied for years by failed efforts to stabilize HIV’s floppy surface protein. But with these detailed structures now in hand, a stable HIV envelope trimer that itself may be useful as a starting point from which to design an immunogen, and a suite of newly identified, conserved viral epitopes, scientists are entering a new phase of vaccine design.

No ordinary antibodies
At the same time that researchers are identifying potential vaccine immunogens to elicit broadly neutralizing antibodies, there is also a renewed focus on understanding how these potent antibody responses develop naturally in chronically HIV-infected individuals. Researchers are trying to determine how the virus or a vaccine immunogen can direct the immune system to make antibodies that recognize the highly conserved HIV epitopes. By tracking the arms race that occurs between virus and immune system in the course of natural infection, researchers have found that neutralizing antibody responses don’t appear until several months after HIV infection occurs, by which time the virus has
VACCINATING AGAINST HIV

The strategies that have been used to develop most of today’s successful vaccines—using attenuated, killed, or inactivated pathogens—don’t work for HIV, which boasts unprecedented genetic variability and a high mutation rate. Researchers are now testing a number of tactics in parallel to protect people against the wide range of HIV subtypes that continue to infect the human population.

CHARACTERIZING ANTIBODY FORMATION

About 25 percent of chronically HIV-infected people naturally produce antibodies that are capable of neutralizing a broad swath of HIV strains by targeting conserved regions (blue, green, and pink) of the virus’s envelope glycoprotein. Researchers are now isolating these so-called broadly neutralizing antibodies and identifying potential vaccine immunogens that may elicit such defenses, a strategy called structure-assisted vaccine discovery. Some groups are experimenting with delivery of multiple immunogens in a specific sequence, to mimic the natural process observed in chronically infected individuals.

MOSAIC ANTIGENS

Computationally derived proteins known as mosaic antigens are created by stitching together stretches of DNA from a range of HIV variants. They can be delivered via viral vector to elicit a cellular immune response, inducing the activity of CD4+ T cells that can boost the potency and durability of broadly neutralizing antibodies and activating cytotoxic CD8+ T cells to help control HIV infection.

PASSIVE IMMUNOPROPHYLAXIS

As an alternative to coaxing the immune system to generate broadly neutralizing antibodies, scientists are also testing whether these molecules can be delivered directly to HIV-positive individuals, an approach called passive immunization. Research is ongoing to increase the potency and/or half-life of these antibodies and to improve function of the antibody’s Fc portion, which interacts with immune cells that lyse infected cells.

GENE TRANSFER

Yet another possibility is to use viral vectors to deliver the genes encoding such antibodies. Researchers are also engineering viral vectors to express mutated versions of the coreceptors that HIV uses to infect host cells, thereby inhibiting viral entry without having to elicit a lengthy and complex antibody maturation process. This approach has proven successful in protecting rhesus macaques from simian/human immunodeficiency virus (SHIV) infection.
Bypassing the immune system
In the absence of immunogens capable of eliciting neutralizing antibodies against HIV, researchers are also exploring whether direct injection of HIV-neutralizing antibodies may be an efficient means of preventing HIV infection. This so-called passive immunization approach is now in Phase 1 clinical trials involving both HIV-positive patients and uninfected volunteers to determine the safety and pharmacokinetics of these vaccines. Early data from monkey studies suggest that such direct injection of broadly neutralizing antibodies may also have therapeutic benefits or even be part of a multifaceted HIV cure strategy.10

Meanwhile, work is underway to optimize the antibodies used for such passive immunization by introducing mutations that increase their potency and/or half-life and by improving the function of the antibody's Fc portion, which can interact with monocytes and natural killer cells to lyse virus-infected cells. There are also plans to study a cocktail of antibodies in passive immunization studies to increase the breadth of activity against HIV.

Antibody responses that can neutralize HIV more broadly—the type researchers seek to elicit with a vaccine—appear only after two or more years of chronic HIV infection.

Cellular immunity
In parallel with studies focused on eliciting broadly neutralizing antibodies, scientists continue to pursue strategies to elicit cell-mediated immune responses against HIV. Induction of CD4+ T cells can boost the potency and durability of broadly neutralizing antibodies and also help activate robust cytotoxic CD8+ T cells aimed at controlling HIV infection. But, as with effective antibodies, such cellular immune strategies face the challenge of high levels of genetic diversity among circulating HIV subtypes.

Bette Korber and colleagues at Los Alamos National Laboratory are designing so-called mosaic antigens to overcome HIV diversity. These are computationally derived proteins created by stitching together genetic sequences from across the entire HIV genome. (See illustration on previous page.) These mosaic anti-
gens, when delivered via viral vectors either alone or in combination with each other or a protein booster component, can provide greater breadth of cellular immune responses against HIV variants and protect against SHIV infection in monkeys. Researchers recently initiated Phase 1 trials of this approach.

An alternative tactic for tackling the variability of HIV is to focus on eliciting cellular immune responses to the most conserved regions of the HIV proteome, an approach championed by Andrew McMichael and Tomas Hanke of Oxford University. Most recently, mosaic antigens that are focused solely on these conserved regions of HIV were designed to optimize coverage of such immunogens across HIV’s global diversity. These conserved mosaic antigens are undergoing preclinical testing.

Lastly, researchers are harnessing the unique qualities of cytomegalovirus (CMV) that evoke robust and broad cellular immune responses by using this virus as a vector for HIV vaccine development. In monkey studies spearheaded by Louis Picker of Oregon Health & Science University, administration of the rhesus form of cytomegalovirus (RhCMV) expressing proteins from simian immunodeficiency virus (SIV), the monkey equivalent of HIV, led to durable control of SIV infection following challenge, including evidence of complete clearance of pathogenic SIV infection in some animals. The precise mechanism for this protection remains unknown, but effector memory T-cell responses appear to play a role. Picker and colleagues are now developing a prototype CMV vector to assess safety and immunogenicity in humans. This approach should advance to clinical testing by 2016.

Beyond HIV

Although many challenges remain, the development and deployment of a safe and effective HIV vaccine is an urgent global health priority. Recent progress is reinvigorating vaccine discovery efforts, and research to better understand HIV and the immune response against it will help to inform broader vaccine efforts. Already, researchers have identified broad and potent neutralizing antibodies against influenza, dengue, hepatitis C, and other complex pathogens. And investigators are applying structure-based vaccine discovery to a wide spectrum of infectious diseases for which vaccines are still needed.

Similarly, new technologies of genetic and immune monitoring and of systems biology, coupled with novel strategies for induction of cellular immune responses, are being applied for development of prophylactic and therapeutic vaccines against infectious diseases and cancers. The prospect of decoding the immune system and unravelling the rules of immunogenicity in humans now offers the potential to usher in a golden age of vaccinology that will relegate HIV and other modern global killers to the same fate as the childhood diseases of the 1950s that are now easily prevented through vaccination.

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References
Contributors

A native of Melbourne, Australia, Genevieve Martin enrolled in a one-year honors program during her medical training at Monash University to study age-related changes in monocytes from HIV-infected women. “When I started this year in HIV medicine, I just became absolutely fascinated, and I thought, this is what I want to do,” Martin says. During her honors research, she first encountered the concept of HIV latency and was struck by “the audacity of the idea.” Martin has just begun doctoral research on the topic with John Frater at the University of Oxford.

Matthew Pace’s high-school fascination with biology continued during his undergraduate years at St. Joseph’s University in his hometown of Philadelphia, where he earned extra money over the summer working in a biochemistry research lab. Eventually, Pace became interested in pathogens and disease, and focused on HIV latency during his doctoral work with Una O’Doherty at the University of Pennsylvania. “It’s much less straightforward . . . because we don’t really understand it yet,” he says of HIV latency. He is now a postdoctoral researcher in Frater’s lab at Oxford.

Working in the AIDS ward of St. Mary’s Hospital in London in the mid-1990s, John Frater, fresh out of medical school, decided to conduct HIV research. He pursued doctoral work with Myra McClure at Imperial College London, investigating the responses of different HIV strains to retroviral medicines, and later probed the human immune response to the virus as a clinical lecturer in the lab of Rodney Phillips at Oxford. Frater remains at Oxford, where he is now a research lecturer in the Nuffield Department of Medicine. He is cofounder and codirector of Collaborative HIV Eradication of Reservoirs: UK Biomedical Research Centres (CHERUB), a collaboration among multiple research institutes that seeks a definitive cure for the disease.

Martin, Pace, and Frater explore the mystery of HIV latency in “Hidden Menace,” page 34.

In the 1970s, Wayne Koff investigated antiviral drugs against influenza while a doctoral student in Vernon Knight’s lab at Baylor College of Medicine. Pursuing what would become a lifelong interest in vaccine development, Koff went on to explore dengue vaccine development and macrophage-mediated suppression of viral infections, a line of inquiry he followed as an assistant professor at the University of Texas MD Anderson Cancer Center in the mid-1980s. In 1986, as the HIV/AIDS epidemic emerged as a public health concern, he undertook a one-year internship at the National Institutes of Health to study the disease, inspiring him to combine his latest research with his long-standing interest in vaccines. After completing his internship, Koff spent four years as NIAID’s branch chief for AIDS vaccine research and development before serving six years as vice president for vaccine research and development at United Biomedical. In 1998, Koff joined the International AIDS Vaccine Initiative (IAVI) as vice president of research and development and is today IAVI’s chief scientific officer. “We’re in a renaissance period in the AIDS vaccine effort,” Koff says. “Years ago, people would ask if an AIDS vaccine would ever be possible. Now, the question is no longer ‘If.’ It’s ‘When?’ ”

Koff explains the science behind designing an HIV/AIDS vaccine in “Defeating the Virus,” page 40.

As a medical student at the University of Ghent in his native Belgium, Peter Piot was told there was no future in infectious disease medicine. Undaunted, he conducted doctoral research in microbiology at the Institute of Tropical Medicine Antwerp, where in 1976 he analyzed the blood sample of a Belgian nun who had died in northern Zaire (today Democratic Republic of the Congo). He and his colleagues isolated a completely new and deadly virus: Ebola. In the early 1980s, Piot began serving on the front lines of the rising HIV/AIDS epidemic in both Antwerp’s gay community and in Africa among heterosexuals. “I never understood why a virus would care about the sexual orientation of a human host,” Piot said in a 2012 interview at the London School of Hygiene and Tropical Medicine, where he has served as director since 2010. Piot’s interest in advocacy led him to found and direct UNAIDS from 1995 to 2008. Piot provides his perspective on the history and future of the science and politics of AIDS in an essay, “Attacking AIDS on Many Fronts” (page 74), based on his new book AIDS: Between Science and Politics.