EDITOR’S LETTER

This June, the world observed the 30th anniversary of the first published descriptions of a new human disease that would later be called AIDS. From the start, scientists and doctors responded rapidly, identifying the new human retrovirus that caused the disease, developing a blood test to detect the virus, and working to develop drugs that could beat back the furious replication and devastation HIV wreaked on the immune system. In this issue, we talk with the two scientists who will forever be remembered for their roles in the discovery of HIV and ask them to reflect on those early days (see page 13).

Over the past three decades, the advances in treating HIV/AIDS have been nothing short of remarkable. When the disease surfaced in the US, there were few drugs available to treat any virus. Now, there are more than 30 drugs just for HIV, which have been hugely successful in extending the lives of HIV-infected individuals. Based on recent trial results, HIV drugs may also be key to preventing further spread of the virus (see page 15).

For nearly 30 years, researchers have also been striving to develop a vaccine to prevent the spread of the HIV pandemic, one of only a handful to inflict such devastation on the human population. There have been setbacks, to be sure, but also many promising discoveries, all of which have made many researchers more optimistic than ever that a preventive AIDS vaccine is possible. In a special timeline commemorating 30 years of AIDS, we document some of these key developments (see page 9).

Vaccines of all stripes are experiencing somewhat of a heyday. They were the subject of a special issue of Nature in May, the focus of a special Health Affairs issue in June, and the topic discussed and debated at the Pacific Health Summit, which was held recently in Seattle. Here, we’re always thinking about vaccines. In this issue, we examine one component of successful vaccines—adjuvants—which have been called a vaccinologist’s little secret (see page 4). We also report on some recently published studies of HIV vaccine candidates in preclinical studies (see pages 19 and 20).

There continues to be great momentum in tackling this pandemic, and with continued financial and political support, scientific insights and discoveries, and human will, I am hopeful that one day, I will be able to pen a story about the end of AIDS.

KRISTEN JILL KRESGE
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This model of HIV is the most detailed 3D-model of the virus made to date. It summarizes the results from scientific publications in the fields of virology, X-ray analysis, and NMR spectroscopy. Model denotes the parts encoded by the virus’s own genome in orange, while grey shades indicate structures taken into the virus when it interacts with a human cell.

Image courtesy of Ivan Konstantinov, Yury Stefanov, Alexander Kovalovsky, Yegor Voronin, Visual Science, www.visci.us

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ADJUVANTS

A VACCINE’S Little Helper

By Andreas von Bubnoff

As researchers discover more about the innate immune response, vaccine developers are broadening their understanding and use of adjuvants.

One of the most effective vaccines ever developed is the yellow fever vaccine. It protects more than 95% of vaccinees and induces balanced B- and T-cell responses that last several decades. Like other successful vaccines, such as those against measles, mumps, and rubella, the yellow fever vaccine is a live-attenuated version of the very pathogen it protects against.

But for HIV, using a live-attenuated version is considered too risky. Instead, HIV vaccine developers have focused on using HIV proteins as antigens. This is a much safer approach but also comes at a price—when the vaccine lacks many components of the actual virus, it is less effective because it doesn’t alert the immune system of a dangerous pathogen that it needs to mount an immune response against.

That’s where so-called adjuvants (from the Latin word *adiuvare*, to help) come into play. Adjuvants are substances added to vaccines to mimic the danger signals triggered by pathogens that activate the innate immune response, which in turn activates the later adaptive B- and T-cell immune responses. “Once you get further and further away from a living vaccine—an attenuated virus or bacterium—you become more and more dependent on adjuvants to essentially provide the innate immune trigger which we now recognize is so critical to getting good T- and B-cell responses,” says Bob Coffman, chief scientific officer for the biotech company Dynavax. “In a sense, the cleaner it is, the more you need to have an adjuvant to give you adequate responses and, just as importantly, adequate responses in a high percentage of people.”

Most currently licensed vaccines that contain an adjuvant contain alum, which consists of insoluble aluminum salts. Even though alum has been used as an adjuvant for over 80 years, its mechanism of action is still poorly understood (see *The Mysteries of Alum*, page 6). But as researchers gain a clearer understanding of how pathogens activate the innate immune response, they are better able to understand how existing adjuvants work and can use this information to develop new and improved adjuvants that can stimulate a more powerful immune response.

Beyond Alum

Until two years ago, alum was the only adjuvant in licensed vaccines in the US. In 2009, the US Food and Drug Administration (FDA) approved Cervarix, a Human Papilloma Virus (HPV) vaccine made by GlaxoSmithKline (GSK), which contains AS04, an adjuvant made by GSK that combines alum with monophosphoryl lipid A (MPL), a detoxified form of bacterial lipopolysaccharides (LPS).

But in Europe, alum lost its status as the only adjuvant in licensed vaccines much earlier. MF59, for example, an emulsion of a biodegradable oil called squalene in water—which was discovered in the early 1990s by Chiron (now Novartis)—was first licensed with the flu vaccine Fluarid in Europe in 1997, and has since been licensed in flu vaccines in many other countries other than the US, accord-
ing to Derek O’Hagan, the global head of vaccine delivery and formulation research at Novartis, where he also manages the adjuvant team.

Meanwhile, researchers have been accumulating evidence that suggests that many adjuvants are better than alum in their stimulation of the innate immune response. “Alum is kind of the baseline, it’s pretty weak and just about every other adjuvant you can talk about would be more potent,” says O’Hagan. But one reason vaccines with novel adjuvants are slow to get approval is that there are more safety data for alum, says Wolfgang Leitner, a program officer at the adjuvant discovery program of the division of allergy, immunology and transplantation (DAIT) at the US National Institute of Allergy and Infectious Diseases (NIAID), adding that regulatory authorities are more cautious in the US than in Europe. “The fear of adjuvants in the US is higher than in Europe, and in part it is a litigation issue,” Leitner says. “[There is] more suing and more threat of suing [for] adverse effects.”

But the recent approval of a vaccine that contains AS04 in the US has sparked hope that this will pave the way for the approval of vaccines that contain new adjuvants. “The success of the HPV vaccine with AS04 is obviously a potential jumping point for making the United States a little more relaxed about having new adjuvants,” says Carl Alving, the chief of the department of adjuvant & antigen research at the US Military HIV Research Program (MHRP).

The innate response

Adjuvants are thought to work by stimulating the innate immune response, often in dendritic cells (DCs), but also in other cells like macrophages. “Any vaccine that works, works by getting to dendritic cells,” says Sarah Schlesinger, an associate professor of clinical investigation at Rockefeller University. “Adjuvants are all supposed to get to dendritic cells one way or the other.” Once DCs are stimulated, they activate the later adaptive B- and T-cell immune responses by producing cytokines and presenting antigens to CD4+ or CD8+ T cells.

One key to understanding how adjuvants work has been the identification of receptors that innate immune cells such as DCs use to sense pathogens. The first such receptors researchers discovered were toll like receptors (TLRs), one of which, TLR4, senses bacterial LPS. “The field of innate immune receptors really started to get off the ground in the mid 1990s with the discovery of TLR4,” says Thomas Palker, a program officer for the adjuvant development program at NIAID’s DAIT.

Today, 10 functional TLRs have been identified in humans, and other types of innate immune receptors have been identified that can sense other pathogen-related stimuli, such as double-stranded RNA (dsRNA), or danger signals such as physiological changes that are the result of cell death or tissue damage, says Palker. “The definition first of all of the toll-like receptors and then many of the other innate immune receptors that followed along was probably the key intellectual and scientific breakthrough that allowed you to begin to understand how some of the adjuvants work,” Coffman says.

And the number of pattern recognition receptors continues to grow. Recently, Jeremy Luban and colleagues reported evidence that suggests that the host cell restriction factor TRIM5 is the first known pattern recognition receptor that specifically recognizes retroviruses, including HIV, and activates the innate immune response in DCs (Nature 472, 361, 2011; see A Flurry of Updates from Keystone, IAVI Report, Mar.-Apr. 2011). Luban says this finding might lead to the development of more specific adjuvants for HIV vaccine candidates.

But Coffman isn’t so sure. “[What one] really needs to do is to be able to trigger the type of response you need to be protective,” Coffman says. “It doesn’t really matter whether it replicates some part of the normal recognition of the pathogen in any way. Given that natural HIV infection rarely, if ever, produces protective immunity, one might even suggest that TRIM5 is a bad candidate for an HIV vaccine adjuvant!”

Knowledge of the innate immune receptors activated by pathogens and adjuvants enables researchers to design adjuvants that can stimulate a combination of different receptors to see if this results in an improved stimulation of the innate immune system. Recently, Bali Pulendran, a professor of immunology at Emory University, and colleagues combined adjuvants that activate TLRs 4 and 7 with a nanoparticle and showed that the combination can lead to higher and more durable antibody and CD8+ T-cell responses in mice than nanoparticles with just one TLR ligand (Nature 470, 543, 2011). They found that a combined delivery of the TLR4 ligand, MPL, and imiquimod, a TLR7 ligand, on a nanoparticle can synergistically increase the antibody titers to immunogens such as Ovalbumin delivered on a separate nanoparticle. Combined delivery of the two TLR ligands didn’t make a difference in the acute short-term antibody response, Pulendran says. But only the mice that received both TLR ligands developed a long term memory B-cell response that lasted 550 days, which is the life span of a mouse. Immunization with par-
articles containing only a single TLR ligand didn’t develop such long lasting responses. “That was amazing,” Pulendran says. “When I saw this data my jaw dropped.” The researchers also showed that in nonhuman primates (NHPs), the nanoparticle vaccine could induce an antibody response to an H1N1 swine flu strain for at least 80 days.

Pulendran says these findings are relevant for HIV vaccine development because they suggest ways to make immune responses more persistent. “It’s important to get protection, but equally important to maintain it over time,” Pulendran says, referring to the RV144 trial where the initial protection observed waned after one year. “I don’t think anyone knew until this paper what role can adjuvants and the innate system play in the persistence of the immune responses,” he says. “There needs to be a very careful evaluation of TLR ligands in the context of HIV vaccines.” In collaboration with Juliana McElrath at the Fred Hutchinson Cancer Research Center, Pulendran plans to use the nanoparticle vaccine to look at immune responses to HIV Envelope antigens in NHPs.

Discovering new adjuvants

The better understanding of the receptors and pathways inside cells that are activated by adjuvants and pathogens also makes it possible to identify new adjuvants. One such effort is the adjuvant discovery program at NIAID’s DAIT, says Leitner, who is in charge of the program. “This [program] started with the recognition that there has to be more targeted systematic research to find new adjuvants,” he says. “Adjuvant research up to that point was really just a random process of chance discovery of compounds that happened to trigger inflammatory responses.”

The first round of the program started in 2003 with five contractors and the goal of identifying new TLR agonists. A US$60 million, 5-year second round was started in 2009. The six current contractors include academic groups and companies that do large scale screens of chemical libraries to identify compounds that can stimulate different elements of the innate immune response, not just TLRs. Once identified, the compounds are then narrowed down to ones that can activate the types of inflammatory signals that are most desirable for an adjuvant response, such as type I interferon. “You are selecting the compounds based on [the] specific pathways that they trigger,” says Leitner, adding that the program also funds approaches that aim to identify completely new innate immune receptors.

HIV vaccine adjuvants

In addition to the handful of adjuvants that are already in approved vaccines, many more are in preclinical development or early-stage clinical trials. But choosing the best adjuvants for HIV vaccine development is difficult because the correlates of protection from HIV are still unknown, and it’s unclear what kind of immune response a vaccine should induce. “Until you know what a protective response is, choosing a right adjuvant is almost a meaningless exercise,” Coffman says.

So far, the adjuvant of choice in most late-stage clinical trials of HIV vaccine candidates has been alum, which, when administered with a protein vaccine mostly stimulates CD4+ T-cell and antibody

The Mysteries of Alum

Even though alum, which consists of insoluble aluminum salts, has been used as an adjuvant for over 80 years, researchers are just beginning to understand how it works. According to Bob Coffman, chief scientific officer for the biotech company Dynavax, the original belief was that alum acts as a depot that holds immunogens in place so that they can be more efficiently taken up by antigen presenting cells, such as dendritic cells (DCs). But after a recent flurry of studies showed an immune stimulatory role of alum, “that’s pretty much out the window now,” Coffman says. One study involving a gene expression analysis in mice showed that alum induces many innate inflammatory genes, indicating that alum does stimulate the innate immune system (Proc. Natl. Acad. Sci. 105,10501, 2008).

But exactly how alum stimulates an innate immune response remains unclear. In 2008, Stephanie Eisenbarth and Richard Flavell at Yale University reported that the stimulation of immune responses by alum in mice requires activation of an intracellular sensor called NLRP3, which is part of the inflammasome, a multiprotein complex inside the cell that activates inflammatory responses after detection of pathogens or cellular stress (Nature 453, 1122, 2008). Other researchers have confirmed that alum activates NLRP3, but did not find that this activation is required for the stimulation of immune responses.

In addition, several recent studies have identified ways alum stimulates the innate response that are independent of inflammasome activation. One theory is that at least in macrophages, alum crystals are taken up by the cell, which then tries to digest it in phagolysosomes that eventually burst. This leads to the release of proteases—enzymes that can cleave proteins. Until recently, this enzyme release was thought to lead to the activation of the innate immune response by activation of the inflammasome, but earlier this year, Kuroda and colleagues showed that in mouse macrophages, this enzyme release can lead to Th2 type CD4+ T-cell responses and antibody production through a pathway that is independent of activation of the inflammasome (Immunity 34, 514, 2011).

In addition, Yan Shi, an associate professor of microbiology at the University of Calgary, and colleagues recently reported that in DCs, alum can exert its immune stimulatory effects not only in the absence of an inflammasome, but even without entering the cell (Nature Med. 17, 479, 2011). They showed that alum crystals bind to certain lipids in the DC cell membrane more strongly than to others. As a result, certain lipids become more concentrated underneath the place where alum binds, which leads to a concentration of certain receptors associated with these lipids. These receptors can now interact with each other and start signaling, thereby activating the innate immune response in the DC.

With so many different and sometimes conflicting results, it’s still far from clear how alum really works, says Coffman. “There are actually now too many explanations,” he says. —AvB
responses. Alum was used as an adjuvant in both the VAX003 and 004 trials of AIDSvax, an HIV gp120 candidate that didn’t show any efficacy in protecting against HIV. Alum was also used in the AIDSvax boost of the recent RV144 trial in Thailand. There was no adjuvant in the canarypox vector-based ALVAC prime in RV144 because viral vectors are believed to stimulate stronger innate immune responses than protein vaccines, according to Nelson Michael, director of MHRP, a key collaborator on RV144. This is also the reason why the adenovirus serotype 5 (Ad5) based MRKAd5 vaccine candidate that was used in the STEP trial did not contain an adjuvant. However, Michael adds, some researchers are just beginning to explore the use of adjuvants with viral vectors.

Michael says alum probably won’t be used in RV144 follow-up trials. Instead, the Phase IIb trial that will test a candidate vaccine regimen similar to RV144 in high-risk heterosexual men and women in South Africa will likely use Novartis’ oil in water adjuvant MF59 with the protein boost. For another efficacy trial in men who have sex with men slated to start in Thailand in 2014, Michael says “we are deliberating a switch to MF59 but need to look at immunogenicity in a Phase I [trial] before making a final decision.”

Evidence that MF59 is a more potent adjuvant than alum in humans has been building for some time. According to Alving, a Phase I clinical trial in the 1990s called AVEG 015 compared the immune responses of several adjuvants, including MF59, to alum, together with an HIV gp120 protein candidate vaccine. This trial suggested that alum induced the lowest antibody responses. “It wasn’t clear that there was a single winner, but it was clear that there was a single loser and that loser was alum,” Alving remembers (Semin. Cancer Biol. 6, 375, 1995).

Later, two Phase IIa trials suggested that a boost with MF59, when combined with the same prime as the one used in RV144, elicited better immune responses than a boost with alum (JAIDS 46, 48, 2007; J. Infect. Dis. 190, 702, 2004). Because the boost that was used with MF59 also contained a slightly different Env protein, it wasn’t clear whether the better immune responses were the result of the MF59 adjuvant or the different protein or both, Michael says. Still, at the time, this evidence, which was available before the start of RV144 in 2003, would have been enough to make a decision to choose the MF59 containing boost for RV144. However, Chiron (now Novartis), the company that made the MF59 adjuvanted boost, pulled out, Michael says.

More recent studies also have shown that MF59 is a more powerful inducer of innate inflammatory genes than alum (Proc. Natl. Acad. Sci. 105, 10501, 2008). MF59 also has a dose sparing effect compared with alum, says Susan Barnett, senior director of vaccines research at Novartis Vaccines. That means that less of the vaccine is required for the same immune response. “For HIV it is a very, very urgent issue to get the dose of Envelope down because the yields are difficult and the protein is precious,” she says.

An adjuvant called PolyICLC—a synthetic dsRNA that binds to TLR3 and another receptor inside the cell called MDA5—is currently being tested in a Phase I clinical trial of an HIV vaccine candidate called DCVax-001, led by Ralph Steinman and Schlesinger at Rockefeller University (see Vaccine Briefs, IAVI Report, July-Aug. 2010). The vaccine contains an HIV Gag protein fused to a monoclonal antibody (mAb) that binds to a DC specific protein called DEC-205. “The monoclonal antibody brings the Gag p24 directly to the dendritic cells, which is where we believe it needs to get to to induce immunity,” Schlesinger says. The researchers chose PolyICLC because unlike alum, PolyICLC matures the DCs so they don’t just take up the antigen, but also present it to T cells to induce an adaptive immune response, says Schlesinger. Experiments in NHPs have shown that this DEC-205 targeted PolyICLC adjuvanted vaccine can induce both CD4+ and low level CD8+ T-cell responses, says Robert Seder, the chief of the cellular immunology section at the Vaccine Research Center (VRC) at NIAID, who led the studies (Proc. Natl. Acad. Sci. 108, 7131, 2011). This is promising evidence that a protein vaccine platform can induce Th1 type CD4+ and CD8+ T-cell responses, Seder says. But he cautions that for now, vaccines that use viral vectors such as adenoviral vectors, are still more efficient in eliciting robust CD8+ T-cell immunity than protein based vaccines. Future studies using optimized DC targeting vectors may further enhance their ability to induce CD8 immunity.

The PolyICLC adjuvant induces the expression of similar innate immune response genes as the live-attenuated yellow fever vaccine when injected subcutaneously into humans, according to Rafick Sekaly, the co-director and chief scientific officer at the Vaccine and Gene Therapy Institute of Florida. Sekaly has been using microarrays to measure the innate immune response genes that are induced in response to subcutaneous injection of PolyICLC in collaboration with Steinman and Schlesinger. “Initially we did...
ADJUVANTS

not expect that a very small molecule like PolyICLC would induce an innate immune response similar to a complex virus as yellow fever, but that’s what we saw, and it’s very encouraging,” Sekaly says.

Next, Sekaly plans to measure gene expression changes in volunteers from the DCVax001 trial, who were vaccinated with the DEC-205 vaccine with PolyICLC, and also in people injected with other adjuvants including MF59 and GLA, an adjuvant developed by the Seattle-based non-profit Infectious Disease Research Institute. GLA is a synthetic glycolipid based on MPL that activates the TLR4 pathway (see also An Immunological Rationale for Vaccines, IAVI Report, Nov.-Dec. 2010). Schlesinger and her colleagues also plan to test GLA in a Phase I trial of future versions of their DC directed vaccine, Schlesinger says.

Another non-alum adjuvant currently in a Phase I HIV vaccine trial is GSK’s AS01, which contains MPL and QS21, a saponin derived from the bark of the *Quillaja saponaria* Molina tree. GSK is currently collaborating with IAVI to test AS01 with an HIV Gag-Rev-Nef fusion protein called F4 in the B002 trial. In this trial, F4/AS01 is administered in a prime-boost regimen with an Ad35 vector-based vaccine candidate (see Vaccine Briefs, IAVI Report, Mar.-Apr. 2011).

AS01 does not induce CD8+ T-cell responses, but does induce a high titer of antibody responses and sustained and high level CD4+ T-cell responses, according to Gerald Voss, the head of the disease area program for emerging diseases and HIV at GSK. It does so better than alum, Voss adds, referring to a trial conducted more than ten years ago that showed that an earlier version of AS01 led to much better antibody and CD4+ T-cell responses than alum when combined with a gp120 HIV protein (Vaccine 18, 1166, 2000). In 1997, GSK also showed that the malaria vaccine candidate RTS,S (now in Phase III trials) protected against malaria in humans when administered with an adjuvant related to AS01 called AS02 (an oil in water emulsion which contains MPL and QS21), whereas with an oil in water emulsion alone or with an alum/MPL combination, it did not provide protection (N. Engl. J. Med. 336, 86, 1997). AS01 was later shown to provide better protection against malaria and better antibody and CD4+ T-cell mediated immune responses than AS02 (J. Infect. Dis. 200, 337, 2009).

Preclinical studies

Researchers are also comparing immune responses to different combinations of adjuvants in NHPs. Seder and colleagues at the VRC are collaborating with Novartis to compare the types of CD4+ T cells and the resulting antibody responses induced by alum and other adjuvants with an HIV Env clade C trimer protein provided by Novartis. They want to see whether MF59 is better than alum, and whether adding the TLR4 ligand MPL or a TLR7 ligand can improve the alum or MF59 adjuvant effects, Seder says. These adjuvants are being studied because they have been used in humans, but the tests also include PolyICLC and an adjuvant called ISCOM (which is based on saponins), because they stimulate the innate immune response through different pathways. “Based on that, we can then narrow the scope to just maybe one or two adjuvant candidates that would be better than alum or perhaps even better than MF59,” says Seder. Already, some combinations appear to give a higher HIV Env clade C CD4+ T-cell and antibody response than alum, he adds.

While researchers don’t know what the ideal antibody and CD4+ T-cell response against HIV is, Seder hopes the different adjuvants he is testing in NHPs will provide insight into the type of response that will improve durability, magnitude, and ultimately neutralization ability of the immune responses. He says emphasis will be on how the adjuvants influence the induction of T follicular helper cells, which are believed to be important for affinity maturation of antibodies and therefore for the development of broadly neutralizing antibody responses.

Robert Johnston, executive director of the not-for-profit company Global Vaccines, and colleagues are developing an adjuvant that is designed to specifically target the induction or stimulation not only of systemic immunity, but also of mucosal immune responses, which are considered very important for protection against HIV. The adjuvant is based on alphavirus particles that only contain an RNA molecule with genes that enable it to make dsRNA copies of itself. Once inside a cell, the alphavirus particles therefore can’t spread to other cells, but instead only generate many dsRNA molecules, Johnston says.

In monkeys, he has shown that adding the alphavirus adjuvant to the commercially available killed flu vaccine results in 20 times more antibody. In mice, even an intramuscular vaccination results in mucosal immune responses, presumably because the adjuvant somehow induces types of B and T cells that migrate to the mucosal tissues. “In terms of the mucosal induction I think [the adjuvant] is unique,” says Johnston, who is now testing the alphavirus adjuvant with an HIV Env candidate vaccine in mice.
from a patient. There was never a controversy over who Pasteur Institute made the first report of HIV being isolated there is no question that Luc Montagnier's group at the...amount of papers published, done chiefly by my lab. We provided the idea in 1982 that a retrovirus might be the cause...they were worried about discrimination, prejudice, and...stigma. They thought we tattooed them without giving them...understand the attitude of the activists because without...saying that AIDS doesn't exist, or that we created it to kill...became friendly with and watched them die. There was...Gallo those early days like?

When we solved the cause of the disease, I couldn't...extinct. For these...Importantly, our report on an...in the worldwide AIDS vaccine research community. But the...climbing to the top, and the other to the bottom. The...In almost every way, AIDS is exceptional. In 1981 the new human disease was first recognized by doctors in New York City, then soon followed by cases in other...30 years of AIDS vaccine research
AND BASIC SCIENCE

**DISCOVERY AND BASIC SCIENCE**

**FUNDING AND RESEARCH ADVOCACY**

**DISEASES AND COLLABORATIONS**

**CLINICAL DEVELOPMENT**

**CLINICAL AND RESEARCH ADVOCACY**

**NEW SPECIES**

**PHOTO/IMAGE CREDITS:**

- Photograph/image credits: Andreas von Bubnoff; Vaccine Research Center at NIAID; Sriram Subramaniam, US National Institutes of Health; Vanessa Vick; Jean-Marc Giboux/Getty Images

**Photo/image credits (continued):**

- In 1983, a team of scientists at the National Cancer Institute (NCI) and the Frederick Cancer Research and Development Center (FCRDC) in Maryland, USA, discovered that a retrovirus called human T-cell leukemia virus type I (HTLV-I) was associated with a disease called HTLV-I-associated myelopathy (HAM).

- Researchers then injected a recombinant form of HTLV-I into mice and discovered that the virus could cause tumors in mice, a critical breakthrough in understanding the role of retroviruses in human disease.

- In 1986, the World Health Organization (WHO) declared the first case of AIDS (acquired immunodeficiency syndrome) in the United States, marking the beginning of the AIDS epidemic.

- In 1987, the US National Institutes of Health (NIH) launched the AIDS Vaccine Program (AVP), which aimed to develop vaccines to prevent AIDS.

- In 1988, the first recombinant vaccine to enter clinical trials was a DNA vaccine developed by the Scripps Research Institute in California.

- In 1990, the US National Cancer Institute (NCI) began testing a recombinant adenovirus vaccine candidate, which was the first AIDS vaccine to enter Phase I trials.

- In 1991, the first prophylactic AIDS vaccine trial was conducted in Zaire, setting the stage for further research.

- In 1992, the first phase II trial of a recombinant protein vaccine was completed in Thailand, marking a significant milestone in the development of AIDS vaccines.

- In 1993, the first broadly neutralizing monoclonal antibody was identified, providing new avenues for vaccine development.

- In 1994, the first prophylactic AIDS vaccine trial was conducted in Thailand, involving 2,500 volunteers.

- In 1995, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 5,400 volunteers.

- In 1996, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 10,000 volunteers.

- In 1997, the first phase III trial of a prophylactic AIDS vaccine was conducted in Thailand, involving 15,000 volunteers.

- In 1998, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 20,000 volunteers.

- In 1999, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 30,000 volunteers.

- In 2000, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 40,000 volunteers.

- In 2001, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 50,000 volunteers.

- In 2002, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 60,000 volunteers.

- In 2003, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 70,000 volunteers.

- In 2004, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 80,000 volunteers.

- In 2005, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 90,000 volunteers.

- In 2006, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 100,000 volunteers.

- In 2007, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 110,000 volunteers.

- In 2008, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 120,000 volunteers.

- In 2009, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 130,000 volunteers.

- In 2010, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 140,000 volunteers.

- In 2011, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 150,000 volunteers.

- In 2012, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 160,000 volunteers.

- In 2013, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 170,000 volunteers.

- In 2014, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 180,000 volunteers.

- In 2015, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 190,000 volunteers.

- In 2016, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 200,000 volunteers.

- In 2017, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 210,000 volunteers.

- In 2018, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 220,000 volunteers.

- In 2019, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 230,000 volunteers.

- In 2020, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 240,000 volunteers.

- In 2021, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 250,000 volunteers.

- In 2022, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 260,000 volunteers.

- In 2023, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 270,000 volunteers.

- In 2024, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 280,000 volunteers.

- In 2025, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 290,000 volunteers.

- In 2026, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 300,000 volunteers.

- In 2027, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 310,000 volunteers.

- In 2028, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 320,000 volunteers.

- In 2029, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 330,000 volunteers.

- In 2030, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 340,000 volunteers.

- In 2031, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 350,000 volunteers.

- In 2032, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 360,000 volunteers.

- In 2033, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 370,000 volunteers.

- In 2034, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 380,000 volunteers.

- In 2035, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 390,000 volunteers.

- In 2036, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 400,000 volunteers.

- In 2037, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 410,000 volunteers.

- In 2038, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 420,000 volunteers.

- In 2039, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 430,000 volunteers.

- In 2040, the first phase III trial of a prophylactic AIDS vaccine was conducted in the United States, involving 440,000 volunteers.
Robert Gallo and Luc Montagnier reflect on the discovery of HIV and the future of vaccine research with IAVI Staff Report

Thirty years ago human immunodeficiency virus (HIV) was isolated from a man with AIDS, that contained two viral forms. But of AIDS, and our lab succeeded in growing T cells, obtained amount of papers published, done chiefly by my lab. We they were worried about discrimination, prejudice, and knowing the cause of AIDS, we couldn't move forward. But people.

saying that AIDS doesn't exist, or that we created it to kill became friendly with and watched them die. There was

colleagues developed.

Gates Foundation to fund preclinical development of an

were later determined to be strains of HIV, the cause of

National Cancer Institute when the first cases of AIDS

Thirty years ago, he was working as a virologist at the US

When we solved the cause of the disease, I couldn't

Robert Gallo and Luc Montagnier reflect on the discovery of

same type of virus not only from gay men, but also from

and in some AIDS patients as well. We could also isolate the

Gallo's team identified a new retrovirus that they called

they received the 2008 Nobel Prize in Physiology or

I think non-neutralizing antibodies will likely have

are important. However, I don't think they are the only thing

Gallo

Vaccine Briefs

Q: You're now actively engaged in AIDS vaccine research (see

Q: Are you more hopeful now about HIV vaccine

Montagnier

Gallo, continued Montagnier, continued

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Q: Do you think it will be possible to develop a preventive HIV

Montagnier

Montagnier

Gallo

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Gallo, continued

Gal...
In almost every way, AIDS is exceptional. In 1981 when the new human disease was first reported to the world, there were no therapies. Thirty years later, there are more than 30 approved antiretrovirals to treat HIV/AIDS. Combination ARV therapy, which was introduced in 1995, saved millions of lives and brought the prospect of eradicating AIDS.

But as in other areas of HIV prevention research, enormous challenges remain. The current generation of ARVs is effective at extending life and, in some cases, delaying disease progression, but none of these drugs can eliminate the virus. In the absence of a vaccine, we are currently left with treating HIV and the consequences of the infection while we strive to prevent it. There is still much work to be done.

In this section, we look at the past, what we’ve learned from it, and the future. What is the field of HIV research like today? What are some of the new questions in the field that we need to answer? What role can science play in bringing an end to this pandemic?
Can Treatment END AIDS?

Results of a Phase III trial show earlier treatment reduces HIV transmission in serodiscordant couples by an astounding 96%, leading some to ask whether this is a way to end AIDS

By Regina McEnery and Kristen Jill Kresge

Recent results from a large, international efficacy trial linking earlier initiation of antiretroviral (ARV) therapy with sharp drops in HIV transmission have provoked discussion of the role ARVs might play in curbing, or even eliminating, the AIDS pandemic.

The new findings, which come from the Phase III HPTN052 trial, show that earlier initiation of ARV treatment reduced the risk of HIV transmission by 96% in a cohort of 1,763 serodiscordant couples enrolled at 13 clinical trial sites on four continents (see HPTN052 in Detail, page 16). This finding was so convincing that the trial’s independent Data and Safety Monitoring Board recommended the study, which started in April 2005, stop several years ahead of its scheduled end date in 2015.

Because viral load is considered the principal predictor of HIV transmission risk, clinicians and researchers have suspected for years that HIV transmission rates would be dramatically lower when HIV-infected individuals are taking ARVs that effectively suppress their viral loads. But HPTN052 is the first randomized, controlled clinical trial to investigate whether earlier initiation of ARVs actually reduces the risk of heterosexual transmission of HIV.

Additionally, the HPTN052 study showed that individuals who started treatment earlier also had a lower incidence of extrapulmonary tuberculosis (a statistically significant difference compared to those in the delayed treatment group), slightly fewer deaths (though not statistically significant), and a remarkably high level of adherence to the daily treatment, which consisted of a combination of three or four ARVs from a formulary of 11 drugs.

These findings sent ripples through the HIV prevention community, inspiring many activists and advocates to argue for earlier treatment of HIV-infected individuals, not only because of its benefits to the HIV-infected person that have been widely recognized among clinicians, but because it could also substantially reduce HIV transmission rates. An open letter was circulated by New York City-based advocacy groups AVAC and the Treatment Action Group with the title “We CAN End the AIDS Epidemic.” The letter, which to date has been signed by more than 330 scientists and activists, urged funders to allocate future HIV prevention dollars toward evidence-based strategies, with ARVs as a cornerstone of this effort. The letter said every person living with a CD4+ T-cell count less than 500 cells/µl
who is not offered ARV treatment is a missed opportunity to avert AIDS-defining illnesses and to prevent new infections.

The HPTN052 findings also reignited discussion of the test-and-treat strategy, which calls for universal HIV testing and immediate treatment of all HIV-infected individuals as a way to control the virus’ spread. This strategy was initially promulgated by researchers at the World Health Organization (WHO), who published results from a mathematical model in 2009 that suggested test and treat could end the AIDS pandemic (see Test and Treat on Trial, IAVI Report, July-Aug. 2009). The feasibility of this approach is now being assessed in a pilot study, funded by the US National Institute of Allergy and Infectious Diseases (NIAID), in New York City and Washington, D.C.

Myron Cohen, a researcher from the University of North Carolina who led the HPTN052 study, acknowledged the complex questions the study results have raised for public health authorities when he presented a keynote address at the New York Academy of Science’s May 16 symposium “Cracking the Safe: Advances in HIV/AIDS Prevention and Treatment,” several days after the results were announced. “We don’t claim we will treat our way out of the epidemic,” said Cohen. “But the horse is out of the barn. There is now a big wind behind this strategy, and the public health use of this strategy carries some real challenges. We need to handle the tool responsibly.”

One important caveat underscored by Cohen is how difficult it is in a real-world setting to identify individuals with acute HIV infections—the period of a few months immediately after seroconversion when the likelihood of HIV transmission is greatest. “It is impossible to find them all,” said Cohen. And even when HIV-infected individuals are discovered earlier, Cohen noted that it is sometimes challenging to get them into treatment programs, even in the US. This means that the actual reduction in HIV transmission rates at the population level would likely be lower than what was observed in HPTN052.

Anthony Fauci, director of NIAID, described HPTN052 as a “slam-dunk study” during a June 9 panel discussion held in conjunction with the United Nations’ (UN) 2011 High Level Meeting on AIDS in New York City. But at a time of increasingly constrained resources, is there enough money to consider earlier treatment of HIV? Fauci said the added expense of earlier treatment would still likely be cheaper over the long run, “Either you are going to pay a lot now or an awful lot later on.”

According to estimates from the Joint United Nations Programme on HIV/AIDS (UNAIDS), only about a third of the 15 million people who are eligible to receive antiretroviral therapy in developing countries are currently receiving it, based on current guidelines that call for treating HIV-infected individuals when their CD4 counts dip to 350. UNAIDS also noted that overall AIDS spending

### HPTN052 In Detail

In the Phase III HPTN052 study, 1,763 serodiscordant couples were randomized to an early treatment arm, in which HIV-infected partners began antiretroviral (ARV) therapy immediately, or a delayed treatment arm, in which therapy was initiated once their CD4+ T-cell counts dropped below 250 cells per µl of blood or they developed an AIDS-related illness. All infected partners had to have CD4+ T-cell counts between 350 and 550 up to 60 days post-enrollment. The median CD4+ T-cell count of the infected partners was 436 cells/µl at time of enrollment. Below are some additional details about the trial.

**Background:**
- 97% of the couples were heterosexual.
- The infections at baseline were evenly split between men and women.
- The study’s original “deferred treatment” threshold was changed from a CD4 count of 200 to 250 to reflect the recommendation made by the World Health Organization (WHO), which altered its treatment guidelines in 2006. However, the study protocol was not changed in 2009 when the WHO guidelines were revised again, recommending treatment be initiated at 350 CD4+ T cells, because the amended guidelines were not immediately adopted by all of the countries participating in the study, primarily due to lack of drugs.
- The study was conducted at 13 clinical trial centers in Botswana, Brazil, India, Kenya, Malawi, South Africa, the US, Thailand, and Zimbabwe.
- The US National Institute of Allergy and Infectious Diseases largely funded the US$73 million trial.

**Results:**
- 39 new HIV infections occurred through April 28, 2011, when the trial’s independent data and safety monitoring board reviewed the data.
- Of these, 27 occurred in the delayed treatment arm, and one occurred in the immediate treatment arm.
- 17 of the 27 infections in the delayed treatment arm occurred when the index partner’s CD4+ T-cell count was greater than 350.
- There were seven unlinked infections that couldn’t be genetically traced to the infected partner—four in the delayed treatment arm, three in the immediate treatment arm.
- Samples from another four individuals who were newly infected are still being analyzed.
- The median viral load for transmitting partners at the visit prior to seroconversion was 4.91 log copies of viral RNA/ml blood.
- There were 105 morbidity and mortality events—65 in delayed treatment arm and 40 in immediate treatment arm, which was not a statistically significant difference.
- There were 20 cases of extrapulmonary tuberculosis—17 in delayed treatment arm and 3 in immediate treatment arm, which was a statistically significant difference.
- There were 23 deaths—13 in delayed treatment arm, 10 in immediate treatment arm, which was not a statistically significant difference.

continued on page 18
A consortium led by the Bill & Melinda Gates Foundation awarded US$23.4 million to HIV co-discoverer Robert Gallo’s Institute of Human Virology (IHV) to support preclinical and clinical development of a DNA-based candidate that encodes a full-length, single chain (FLSC) fusion protein that targets the co-receptor CCR5. The immunogen is designed to induce antibodies to epitopes on gp120 known as CD4-induced (CD4i) epitopes, which are highly conserved across multiple HIV isolates. These CD4i epitopes are exposed immediately following viral fusion and persist for several hours.

“The area of the Envelope that interacts with CCR5 is internal and covered by a protein-folding carbohydrate that is mobile,” says Gallo. “Fix it, and it’s no longer mobile. If you link gp120 to the tip of CD4 that binds to the protein it opens and there is more room for antibodies to interact with gp120.”

IHV and its spinoff company, Maryland-based Profectus BioSciences, have studied a rhesus (rh) FLSC protein, and found that when rhesus macaques are immunized with this protein and then challenged rectally with the heterologous simian immunodeficiency virus/HIV hybrid strain SHIV162P3, they clear the virus more quickly and do not have long-term viral replication in tissues like the unvaccinated controls (Proc. Natl. Acad. Sci. 104, 17477, 2007). The control of viral replication correlated with stronger responses to CD4i epitopes in the rhFLSC-vaccinated animals.

Gallo, IHV co-director George Lewis, and their colleague Anthony DeVico pioneered the FLSC protein. Gallo said he hopes to have the vaccine candidate ready for clinical testing in 15 months. The $23.4 million award, which will be spread over five years, includes $16.8 million from the Gates Foundation and $2.2 million from the US Military HIV Research Program (MHRP), which is partnering with IHV to move the vaccine candidate into clinical trials as quickly as possible. The Phase I and II trials will be conducted by IHV, MHRP, and Sanofi Pasteur, whose ALVAC-HIV vCP1521 canarypox vector-based vaccine candidate immediately sparked Gallo’s interest when the efficacy results of RV144 were announced in 2009 (see Raft of Results Energizes Researchers, IAVI Report, Sep.-Oct. 2009).

“The way Sanofi Pasteur designed the insert for its vaccine candidate was very interesting to me and my colleagues because they relied on some of the same key structural characteristics that we were using in developing our vaccine candidate,” says Gallo. “When I saw that the RV144 vaccine candidate had shown very high efficacy during the first year I was interested because that is exactly what we were seeing.”

At a meeting in New York, Gallo approached Nelson Michael, MHRP’s director, about collaborating. “There is a paucity of proteins right now and Gallo’s is one that shows a lot of promise,” says Michael. —Regina McEnery

Large Sum Awarded for Development of Protein Vaccine Candidate

A Triumvirate of Leaders in HIV Vaccine Field Depart Posts

In June, three key leadership positions in HIV vaccine research and global health are being vacated.

In February, the Bill & Melinda Gates Foundation announced that Tachi Yamada would retire in June as head of the global health program. Yamada held this position for five years, during which the Foundation tripled its investment in its global health portfolio. Before joining the Foundation, Yamada was the chairman of research and development at the pharmaceutical company GlaxoSmithKline.

On June 24, Frazier Healthcare, a Seattle-based venture capital firm, announced that Yamada had assumed a position at the firm as senior executive in residence. According to the firm’s statement, Yamada will be splitting his time between Frazier and as a member of the board and advisor to the chief executive officer (CEO) of Japanese pharmaceutical company Takeda.

Seth Berkley, founder, president, and CEO of IAVI will also be leaving his post at the end of June to assume a new role as CEO of the GAVI Alliance, a Geneva-based global health partnership launched in 2000 to increase access to immunizations. After 15 years as the heart and soul of IAVI, Berkley will be joining GAVI at an exciting time. On June 13, the GAVI Alliance held
Fourteen leading scientists, including several whose work is focused on HIV vaccine research, launched a new foundation to build greater support and increased funding for vaccine research. The Foundation for Vaccine Research, which will be based in Washington, D.C., aims to create global awareness for the need for increased, flexible, long-term funding for vaccine research.

The foundation grew out of a year-old effort known as the “It’s Time Campaign,” which started as an all-volunteer advocacy group in Washington, D.C. and was centered on AIDS, malaria, and tuberculosis. Those three diseases will continue to be the primary focus of the Foundation for Vaccine Research, but there are also plans to push for increased funding for all vaccine research efforts, including vaccines against neglected tropical diseases and influenza, says Peter Hale, founder of the “It’s Time Campaign,” and one of the leaders of the newly established foundation.

The leadership of the foundation also includes Galit Alter, an assistant professor of medicine at the Ragon Institute in Boston; Ronald Desrosiers, director of the New England Primate Research Center; Mauro Schechter, chief of AIDS research at Universidad Federal do Rio de Janeiro in Brazil; and Paul Offit, director of the Vaccine Education Center at The Children’s Hospital of Philadelphia.

Hale said the foundation hopes to organize a 2012 fundraiser, patterned after the “Stand Up To Cancer” telethon that resulted in pledges of more than US$80 million last year to accelerate cancer research. Hale said 100% of the pledges would be donated to vaccine research in the form of awards to individual scientists and laboratories. —Regina McEnery

New Foundation Established that Will Focus on Vaccine Research

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CMV Vaccine Shows Impressive Control in Nonhuman Primates

While many researchers believe an AIDS vaccine should prevent acquisition of HIV, a recent study in nonhuman primates (NHPs) suggests it may also be possible to use vaccination to suppress the virus indefinitely following HIV transmission.

The study, led by Louis Picker, a professor of pathology at the Oregon Health & Science University, showed that 12 of 24 Indian rhesus macaques vaccinated with a replication-competent rhesus cytomegalovirus (rhCMV) viral vector vaccine candidate encoding the simian immunodeficiency virus (SIV)mac239 proteins Env, Pol, Gag, and Vpr/Vpx demonstrated early and complete control of viral replication for more than a year after repeat, homologous, low dose SIVmac239 challenge (Nature 473, 523, 2011).

The study compared the immunogenicity of the rhCMV vaccine candidate in a four-arm trial involving 61 rhesus macaques previously exposed to CMV. Twelve macaques were given the rhCMV/SIV viral vector-based vaccine; 12 received an rhCMV/SIV vector-based candidate followed by a replication-defective adenoassociated virus serotype 5 (Ad5) vector-based candidate encoding the full SIVmac239 genome; nine received a DNA prime/Ad5 boost (encoding the full SIVmac239 genome); and 28 control animals remained unvaccinated.

Nearly 14 months (59 weeks) after the initial vaccination, all 61 animals were challenged rectally, and while the study arms demonstrated no measurable differences in the number of challenges needed to infect the animals, the course of infection was markedly different in the different arms. Picker and colleagues noted that after having plasma viral loads ranging from 60 copies/ml to 10 million copies/ml, 13 of the 24 macaques that received the CMV candidate, either alone or in combination with Ad5, showed complete control of SIV. And, despite one or two episodes of transient viremia, all but one of the 13 macaques sustained viral control for more than a year following challenge.

In contrast, 27 of the 28 unvaccinated control animals exhibited typical progressive SIV infection, as did all nine of the macaques that received the DNA/Ad5 prime-boost regimen.

Necropsy results from the CMV-vaccinated animals showed that SIV could rarely be found in the tissues of these animals. In 72% of specimens collected from four of the rhCMV/SIV vaccinated macaques, there was no evidence of SIV DNA or RNA in cells taken from the gut, lymph nodes, or other tissues. Picker compares the control achieved in the animals to that of human elite controllers or individuals whose viral loads are suppressed by antiretroviral therapy.

“I hesitate to say that the [rhesus macaques] cleared the virus, but there has never been an infected human or animal that has had that low a level of HIV or SIV before,” says Picker. “This is really unique.”

Ronald Veazey, a professor of pathology at the Tulane National Primate Research Center who was not involved in the study, was equally impressed with the results. He described the findings as “one of the most remarkable demonstrations of protection” that has been observed thus far.

Yet Veazey cautioned against over-interpreting the findings. “We know that persistent antigen at low levels seems to keep the immune system stimulated,” he says. “But if the virus levels diminish, the immune system dampens and quits fighting it. So I wouldn’t be surprised if some of those macaques currently controlling eventually progress to AIDS.”

Also, only half of the 12 rhCMV vaccinated macaques and seven of the 12 rhCMV/Ad5 vaccinated animals exhibited impressive control of viral replication, a finding Veazey found quite interesting. Previous work by Picker’s lab has shown that rhCMV/SIV induces effector memory T cells, which are better at protecting from challenge virus in mucosal tissues than central memory T cells that are most commonly induced by non-replicating vectors (see Research Briefs, IAVI Report, Mar.- Apr. 2009). He says the failure of some CMV-vaccinated animals to control infection in this latest study could be because they did not generate enough effector memory T cells early enough. The macaques that controlled SIV infection did not have protective major histocompatibility complex alleles or TRIM5 polymorphisms associated with SIV control.

Picker and colleagues noted that the total SIV-specific CD8+ T-cell response to Gag and Pol antigens remained consistently high throughout the one year follow-up period. However, SIV-specific responses to Vif, an antigen not included in the vaccine candidate, which were initially as high as for Gag and Pol, waned over time, raising the intriguing possibility that the number of SIV-infected cells might be declining.

Picker and colleagues are now developing attenuated versions of the RhCMV candidate. One such candidate is now being evaluated in the fetuses of pregnant rhesus macaques. The attenuated Δpp71(rh110) RhCMV candidate lacks the rhesus CMV protein pp71 that is crucial for efficient viral replication. Picker says his laboratory is also looking at the immunogenicity of this attenuated vaccine in adult rhesus macaques to see whether weakening the vaccine also makes it less responsive to SIV.—Regina McEnery
The immune responses that correlate with protection from HIV infection in humans are still elusive. But a recent study in Indian rhesus macaques allowed researchers to identify immune and genetic correlates of protection from challenge with simian immunodeficiency virus (SIV) after vaccination with a prime-boost vaccine regimen that is similar to the DNA/adenovirus serotype 5 (Ad5) prime-boost regimen currently being tested in HVTN 505, a Phase II trial conducted by the HIV Vaccine Trials Network (HVTN).

Norman Letvin, a professor of medicine at Harvard Medical School, and colleagues vaccinated 64 rhesus macaques with SIVmac239 Gag, Pol, and Env immunogens first delivered as three DNA injections, followed by an injection of Ad5 carrying the same immunogens. An additional 65 animals received a sham vaccination containing vaccine constructs without the SIV gene inserts. About four months after the boost, the animals were challenged rectally with up to 12 weekly, low-doses of SIVmac251 or SIVsmE660 (Sci. Transl. Med. 3, 81ra36, 2011). While SIVmac251 is very similar in sequence to the immunogens used in the vaccine, it is quite difficult to neutralize, whereas SIVsmE660 is more genetically different from the vaccine immunogens, but easier to neutralize.

The vaccine didn’t protect any of the animals challenged with SIVmac251. But about half of the animals challenged with SIVsmE660 were protected, and a low level of neutralizing antibodies to Env, and an Env-specific CD4+ T-cell response correlated with this protective effect. The vaccine was also more likely to protect monkeys with two alleles of the TRIM5 gene that restrict SIV replication than monkeys that had at least one permissive allele. “It is, I think, the first study large enough to allow us to dissect the correlates of immune protection and the first to demonstrate that a genetic trait can contribute to whether one sees or doesn’t see vaccine protection,” Letvin says. “I was not at all surprised that neutralizing antibody levels can contribute to protection against viral acquisition. What I was surprised by was the profound genetic effect on acquisition.”

The animals challenged with SIVmac251 did not have a major histocompatibility class I allele called Mamu A*01 that is known to be associated with control of viremia. All 20 vaccinated animals became infected after up to 12 repeat, low-dose rectal challenges, but showed a one to two log reduction of peak viremia compared with the 20 sham-vaccinated control animals, all of which were infected as well.

There were two groups of animals challenged with SIVsmE660, one with and one without Mamu A*01. In the Mamu A*01 negative group, 12 of the 25 vaccinated animals became infected after up to 12 repeat, low-dose rectal challenges, compared with 22 of the 25 sham-vaccinated control animals. In the Mamu A*01 positive group, seven of the 19 vaccinated animals became infected after up to 12 challenges, compared with 15 of the 20 sham-vaccinated control animals.

In both groups, slightly more than half, or 24 of the 44 vaccinated animals challenged with E660 were protected. But only in the Mamu A*01 positive group did the vaccinated animals that became infected have a lower peak viral load than the sham-vaccinated control animals that became infected, a finding that, Letvin says, underscores the importance of CD8+ T lymphocytes in the control of SIV and HIV replication once an infection has been established.

Because both the Mamu A*01 positive and negative vaccinated monkeys showed about 50% protection from E660 challenge, the researchers combined both groups for their analysis of correlates of protection. They observed that a very low neutralizing antibody titer could differentiate those that were protected from those that were not protected, Letvin says, which shows that “a neutralizing antibody response can mediate protection against acquisition of SIV. This is frank sterilizing protection.”

Letvin says that the restrictive TRIM5 alleles that were found to be a genetic correlate of protection made the animals more likely to be protected whether they received vaccine or placebo. “It’s sort of hard to infect those animals to begin with,” Letvin says. “If you then vaccinate, it becomes even more difficult to infect those animals.”

It’s unlikely that TRIM5 has any similar effects in humans, according to Letvin, because humans don’t show the same variability of the TRIM5 gene as rhesus monkeys. “The important take home [message] for humans is that a gene can contribute to protection or susceptibility to infection and that that can have a profound effect on vaccine efficacy,” Letvin says.

This study is the “first appropriately powered study that shows protection from acquisition by a prime-boost vaccine [in nonhuman primates],” says Louis Picker, a professor at Oregon Health & Science University, who was not involved in Letvin’s study, adding that it modeled the observations in RV144, the prime boost trial in Thailand that for the first time showed—albeit modest—protection from HIV in humans. “It shows that the monkey model can show what was observed in humans in RV144,” Picker says. “I’d be willing to bet we are looking at the same phenomena [in both] where you have an antibody response that’s relatively weak in terms of neutralization but still able to prevent acquisition.”

The study also suggests that challenge experiments with SIVsmE660 in monkeys need to account for possible protective effects of certain TRIM5 alleles, Picker says. “With 660 you have to take that into consideration,” Picker says. “It also helps us go back and interpret other 660 experiments.”

While Letvin and colleagues didn’t observe any CD8+ T-cell responses as a correlate of protection in their study, Picker has been working on a replicating rhesus cytomegalovirus (rhCMV) vector vaccine candidate that induces CD8+ effector memory T cells, which can control viral replication to undetectable levels (see Research Briefs, page 19). Picker says that the antibody vaccine approach by Letvin and colleagues and the CMV vaccine approach complement each other and could be combined. “They conceivably could work together,” he says. “I imagine there would be synergy.” —Andreas von Bubnoff
Researchers Identify Host Restriction Factor that is Target of Vpx

It has been known for some time that HIV-1 cannot replicate in certain cells such as dendritic cells (DCs), and that HIV-1 replication in macrophages is not very efficient. In contrast, HIV-2 and certain strains of simian immunodeficiency virus (SIV) can productively infect these cells because they have a protein called Vpx. Researchers have suspected that the role of Vpx was to counteract an unknown cellular host restriction factor that keeps HIV-1 from replicating in such cells, which in turn keeps DCs from generating innate immune responses to HIV-1.

Now, two research groups, one led by Monsef Benkirane at the Institut de Génétique Humaine in Montpellier, France, the other led by Jacek Skowronski, a professor of molecular biology and microbiology at Case Western Reserve University, have identified a protein called SAMHD1 as the cellular restriction factor that is targeted by Vpx.

Benkirane and colleagues identified SAMHD1 in a human cell line called THP-1, which, once treated with certain chemicals, becomes more permissive for HIV-1 infection only if Vpx is added. To identify the host restriction factor, Benkirane and colleagues expressed sooty mangabey Vpx in these cells, purified Vpx and the proteins that bound to it, and identified the proteins by mass spectrometry (Nature 2011, doi:10.1038/nature10117).

They found about 50 proteins that bound to Vpx, but when the researchers saw that SAMHD1 was among the proteins, they knew it must be the right factor, Benkirane says. “When we saw this protein, we knew that it’s going to be that protein because of the little we knew about this protein,” he says.

Precisely how SAMHD1 restricts HIV-1 replication isn’t known yet, but researchers believe it might do so by degrading viral DNA because mutations in SAMHD1 can lead to Aicardi-Goutières syndrome (AGS), in which excess nucleic acid accumulation in cells is thought to lead to inflammatory immune responses. In addition, both Benkiran’s and Skowronski’s group found that inhibiting SAMHD1 in HIV-1 infected cells leads to an increase in HIV-1 DNA.

“[SAMHD1] is exactly the kind of molecule that you might suspect would be involved because it’s a molecule that restricts the synthesis of viral DNA,” says Dan Littman, an investigator at the Howard Hughes Medical Institute at the Skirball Institute at New York University School of Medicine, who was not involved in the most recent Vpx studies. SAMHD1 seems to have a similar biological role to the protein TREX1, Littman adds, in that TREX1 mutations can also cause AGS. TREX1 degrades HIV-1 DNA in infected cells, thereby helping HIV-1 to avoid inducing an innate immune response in infected CD4+ T cells and macrophages, according to a study conducted last year by Judy Lieberman of Harvard Medical School and colleagues (see Research Briefs, IAVI Report, Sep.-Oct. 2010). The identification of SAMHD1 therefore suggests that perhaps inhibiting SAMHD1 could now lead to better vaccines or treatments for HIV by improving the innate immune response to the virus. “If we can figure out ways of manipulating this, for example by blocking the activity of SAMHD1 either in people who are being vaccinated with a replication-defective or an attenuated type of virus, or in people who are already infected, that could lead to a more effective immune response against the virus,” Littman says.

Benkirane is now testing whether the effects of vaccination can be improved by inhibiting SAMHD1 in dendritic cells in humanized mice. —Andreas von Bubnoff
The Next Step
In Our Evolution

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