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ANTIBODIES: BEYOND NEUTRALIZATION

## **NIPPING HIV IN THE BUD**



**PLUS**

**CONFERENCE UPDATES:**

**Keystone Symposia on HIV Pathogenesis**

**17th Conference on Retroviruses and Opportunistic Infections**



## EDITOR'S LETTER

W

WE'VE ENTERED THE FOURTH DECADE in the battle against HIV/AIDS. The first was indelibly marked by the quick and certain death HIV infection brought. The landmark discovery in the second decade of HIV was the introduction of highly active antiretroviral therapy, the combination of drugs that rescued many HIV-infected people from the brink of death. In the third decade, considerable progress was made in delivering these life-saving antiretrovirals (ARVs) to millions of people in need, particularly in low- and middle-income countries.

What will be the most important accomplishment of this decade? Many hope it will be significant progress in developing new methods to protect people against HIV.

AIDS vaccine researchers, who were buoyed last year by the first evidence of vaccine-induced protection in humans and the discovery of several new broadly neutralizing antibodies against HIV, will likely reap important new insights in this decade that could lead to the development of an effective preventive vaccine. Some of these insights may relate to the role of innate immunity in protection against HIV, and specifically the non-neutralizing functions of antibodies that recruit innate immune responses (see *Antibodies: Beyond Neutralization*, page 8). Also, the next few years will bring a flurry of results from studies of pre-exposure prophylaxis (PrEP), an HIV prevention strategy that entails administering ARVs either orally or in a microbicide gel to uninfected people in an attempt to protect them against infection.

HIV vaccine research and PrEP, along with test and treat—universal testing and immediate treatment of infected individuals—were among the main topics that headlined the 17th Conference on Retroviruses and Opportunistic Infections that took place in February (see *Prevent and Conquer*, page 13). Many other basic research discoveries were showcased in January at the annual Keystone Symposia on HIV Biology and Pathogenesis (see *On the Scientific Trail in Santa Fe*, page 4).

More than ever, researchers are focusing on preventing the spread of the virus, optimizing treatment for individuals who are already infected, and eventually finding a cure for HIV. Given this, the fourth decade of the pandemic should bring us even closer to the ultimate goal—conquering AIDS.



KRISTEN JILL KRESGE



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The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996 and operational in 25 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI's financial and in-kind supporters include the Alfred P. Sloan Foundation, the Bill & Melinda Gates Foundation, the Foundation for the National Institutes of Health, The John D. Evans Foundation, The New York Community Trust, the James B. Pendleton Charitable Trust, The Rockefeller Foundation, The Starr Foundation, The William and Flora Hewlett Foundation; the Governments of Canada, Denmark, India, Ireland, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government, the European Union as well as The City of New York, Economic Development Corporation; multilateral organizations such as The World Bank; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, Google Inc., Henry Schein, Inc., Merck & Co., Inc., Pfizer Inc, and Thermo Fisher Scientific Inc.; leading AIDS charities such as Broadway Cares/Equity Fights AIDS and Until There's A Cure Foundation; other private donors such as The Haas Trusts; and many generous individuals from around the world. For more information, see [www.iavi.org](http://www.iavi.org).

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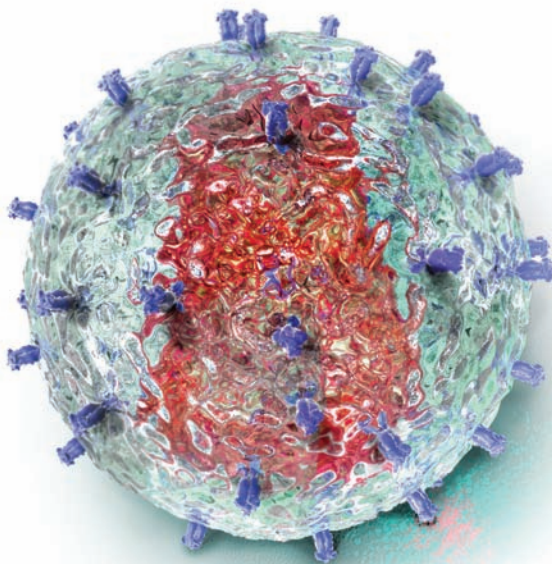
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# IAVIReport

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## [ ON THE COVER ]

Existing models of HIV-1 budding had to be revised based on recent structural data obtained by electron tomography. These data showed that released immature viruses have a different structure and composition than the so-called "late" budding sites previously thought to be viral precursors. This suggests that the structure of the released virion is determined by a kinetic competition between assembly and release. Image is a montage of structures of immature HIV-1 particles and of viral budding sites on infected T cells. Membrane is rendered in semitransparent blue, the viral Gag protein in red.

For details, see Carlson LA, Briggs JA, Glass B, Riches JD, Simon MN, Johnson MC, Müller B, Grünewald K, Kräusslich HG, *Cell Host & Microbe* 2008 Dec 11;4(6):592-9.

# On the Scientific Trail In Santa Fe

A walk through some of the pioneering research presented at the annual Keystone Symposia on HIV Biology and Pathogenesis

By Regina McEney

EVER SINCE HIV WAS DISCOVERED, researchers have been probing the retrovirus' life cycle. Some of their recent progress was highlighted during the annual Keystone Symposia on HIV Biology and Pathogenesis, which was held from January 12-17 in Santa Fe, New Mexico. Just an hour's drive from the Los Alamos National Laboratory where the so-called Manhattan Project coordinated development of the first nuclear weapons, nearly 300 HIV scientists gathered to discuss research that they hope will one day detonate the virus.

An assortment of findings were presented that are shedding light on the drivers of immune activation, components and mechanisms of innate immunity, as well as the mechanisms of HIV transmission.

Some of these advances have been a long time in coming. A major highlight of the meeting was the unveiling of a crystal structure of an integrase protein from the recombinant prototype foamy virus (PFV), a nonpathogenic retrovirus (see Figure 1). Scientists attending the conference described the research as a *tour de force*.

## A shadowy enzyme

Researchers succeeded 21 years ago in building crystal structures of an HIV protease complex. They followed suit nine years later with HIV reverse transcriptase. It is the third canonical retroviral enzyme integrase that has been the proverbial black box scientists have been unable to decode. Although structures of several HIV integrase fragments have been determined, it was far

from obvious how they could be assembled together and how the full-length protein engages viral and human DNAs. While crystallization of full-length HIV integrase remains an elusive target, Peter Cherepanov, a professor at Imperial College London, and his team took a giant step forward last month when their needle-in-a-haystack search produced a crystal structure of PFV integrase bound to its DNA ends, a complex known as the pre-integration complex (PIC), or intasome (see Figure 2). Formation of the PIC is the stage immediately before reverse transcriptase (RT) copies viral RNA genomes into double-stranded complementary DNA (cDNA), which then get integrated into the host's DNA (*Nature* 2010, doi:10.1038/nature08784). Integration is the point of no return in HIV infection because once the viral genome is inserted into the host's DNA you can't get rid of it.

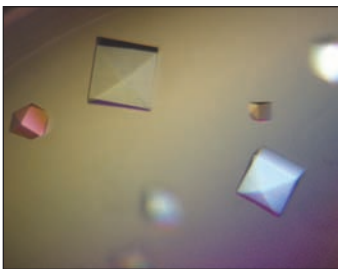
Cherepanov says he will never forget the day his team identified the successful crystal structure at a resolution of three Angstrom. The team had to set up more than 40,000 crystallization trials, and most of the crystals they succeeded to grow were not of sufficient quality for structure determination. "The rest was quick," said Cherepanov. "After years of preparatory work we solved the structure within two weeks." Cherepanov was actually on the London Tube, heading back to his lab, when he got his first glimpse of the partially solved structure. "It was hard not to scream," he said.

Cherepanov's lab used PFV to grow the integrase crystal because it was considered a good proxy for HIV and because HIV integrase had a

FIGURE 1

### Integrase Finally Crystallizes

Crystals of the prototype foamy virus (PFV) integrase-DNA complex that were used to determine its three-dimensional structure.



Courtesy of Peter Cherepanov at Imperial College London

well-deserved reputation for “misbehaving.” The protein’s inherently poor solubility had made it impossible to concentrate HIV integrase to the degree required for crystal formation.

According to Cherepanov, retroviral integrase proteins are very similar. They share domain organization and even sequence homology. “Here, taking two similar proteins with identical function, we can be quite certain their overall structures will be very similar.” Furthermore, Cherepanov says that the amino acid sequence within the active sites of integrase in HIV and PFV is also similar.

When Cherepanov’s team soaked the PFV integrase crystal in solutions of the HIV integrase-inhibiting drugs raltegravir and elvitegravir, they were able to observe, for the first time, how these ARVs bind to and inactivate integrase. “Using the PVF structure as a template, it is now relatively straightforward to generate reliable models for the HIV intasome, which will help improve the design of drugs that target HIV integrase.”

### On high alert

The detrimental effects of chronic immune activation, which is the result of HIV infection, have become increasingly clear (see *Everything from Cause to Cure, IAVI Report*, July-Aug. 2009). Less clear are the biological components that drive immune activation in HIV-infected individuals.

Daniel Douek, chief of the Human Immunology Section at the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases (NIAID), has looked at whether elevated levels of biological products associated with microbial translocation—the leakage of endotoxins and other microbial products across the gastrointestinal barrier and into systemic circulation—is a key driver of immune activation, and therefore disease progression. In previous experiments, Douek’s team found elevated levels of bacterial lipopolysaccharide (LPS)—a component of Gram negative bacterial cell walls that stimulates toll-like receptors on macrophages and dendritic cells (DCs)—in HIV-infected individuals compared to HIV-uninfected individuals, as well as elevated levels of other bacterial markers such as 16S ribosomal RNA and soluble CD14 (sCD14; see *Down, But Not Out, IAVI Report*, March-April 2008). Douek’s team believes microbial translocation occurs because of HIV-inflicted damage to the epithelium and the immune component of the gut.

At Keystone, Douek presented data from 700 HIV-infected individuals that were part of the

SMART study. In the SMART study, nearly 5,500 HIV-infected individuals with CD4<sup>+</sup> T-cell counts of at least 350 were randomly assigned to either a viral suppression arm that offered continuous use of antiretroviral therapy for the duration of the trial, or a drug conservation arm, in which the administration of ARVs was deferred until the HIV-infected individual’s CD4<sup>+</sup> T-cell counts dropped below 250, at which point ARVs were initiated and continued until their CD4<sup>+</sup> T-cell counts increased to more than 350. The cycle of intermittent therapy continued in the drug conservation arm for the duration of the trial. The SMART study was halted in 2006 after data showed a significantly increased risk of disease or death from any cause in those undergoing intermittent treatment (*N. Engl. J. Med.* 355, 2283, 2006).

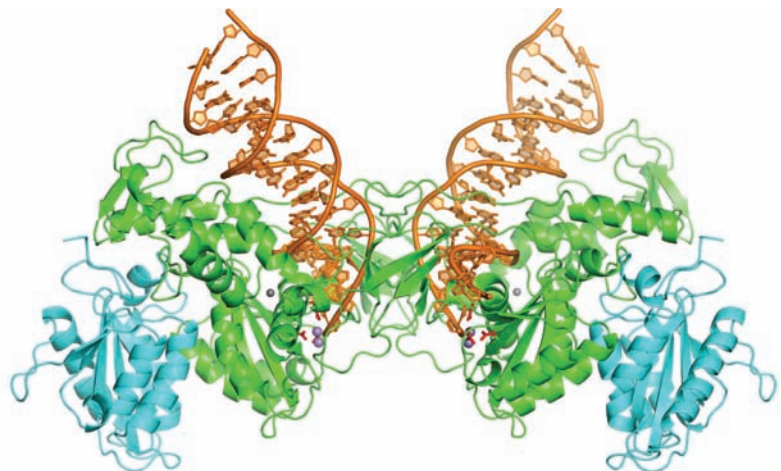
In the SMART study sub-analysis, Douek found that HIV-infected individuals were more likely to have elevated levels of LPS, 16S ribosomal RNA, and sCD14 when compared with a control group of unmatched HIV-uninfected individuals.

“We found plasma sCD14 levels were a predictor of death both in treated and untreated [HIV-infected] individuals, independent of other markers of inflammation and independent of viral load,” said Douek. “We believe these microbial products contribute to immune activation.”

FIGURE 2

### Crystallized Integrase Protein

An overview of the full-length structure of retroviral integrase from a prototype foamy virus, shown perpendicular to the crystallographic two-fold axis. Viral DNA strands are shown in brown. Subunits of the integrase tetramer engaged with viral DNA are blue and green. Image provided by Peter Cherepanov of Imperial College London.



## [ NEW ANTIVIRAL TARGETS ]

The keynote address at the Keystone Symposia on HIV Biology and Pathogenesis was given by Columbia University molecular biologist Steve Goff, who noted how astonishingly complex HIV is compared to other microorganisms. While HIV contains just nine genes that code for 15 proteins, Goff said the virus may exploit as many as 200 human proteins, known as HIV dependency factors (HDFs), to survive and replicate (*Science* 319, 921, 2008). “It’s a nightmare,” said Goff, calling attention to a slide that mapped the vast array of HDFs that conspire in a broad array of cellular functions that begin with the virus binding to CD4<sup>+</sup> T cells, and end with the budding of a new virus.

To disrupt these cellular processes, Goff’s laboratory combs through gene fragments that can potentially interfere with HIV. But while the list of antiviral genes continues to grow, the big question is whether scientists will be able to harness their potential to either prevent or treat disease. —RM

His group also found higher levels of a fourth biomarker—intestinal fatty acid binding protein (I-FABP)—in plasma. I-FABP, which is generally confined to the lower intestine, is associated with a loss of epithelial cell integrity, usually in response to blood supply restriction or tissue injury. “We think I-FABP may be a nice way of measuring gut damage rather than through gut biopsies,” said Douek.

Douek said they were unable to compare whether the time of initiation of highly active antiretroviral therapy (HAART) impacted the levels of LPS, sCD14, or I-FABP. Nonetheless, he said the results underscore the fact that immune activation is undesirable. And although ARVs can successfully suppress viral replication, this may not be enough to eliminate immune activation. “When you reduce the virus to very, very low levels, you don’t fix the problem completely. You can have immune activation, all this evil going on, even when there is no [detectable] virus,” said Douek.

But Douek cautioned not to trivialize the role of the virus in this chain reaction of events. “None of this happens without the virus causing damage in the first place,” said Douek. “You don’t get AIDS from immune activation unless you have HIV.”

## Aging on HAART

Another one of the perils of chronic immune activation in HIV-infected individuals, it seems, may be accelerated aging. Previous data indicate that HIV-infected individuals on long-term HAART are at greater risk of non-HIV related conditions than age-matched HIV-uninfected individuals. Many of these complications are similar to those observed in the aging population, and include cardiovascular, liver, and kidney disease, as well as osteoporosis and non-HIV related cancers. While antiretroviral drug toxicity is considered to be one reason for the early manifestation of some of these premature age-related conditions, researchers believe there are other factors, including immunologic dysfunction and inflammation that persists even during suppressive antiretroviral therapy, that are driving onset of age-related diseases in HIV-infected individuals.

Steven Deeks, a professor of medicine at the University of California, San Francisco, analyzed an array of immunological markers associated with aging or with cardiovascular disease, which occurs more frequently as people age, in 100 females from the Women’s Interagency HIV Study (WIHS) and 300 HIV-infected men and women from the SCOPE study, and compared them to age-matched uninfected individuals.

The WIHS analysis showed that levels of immunosenescent T cells, as measured by their lack of CD28 expression, was higher in untreated and treated HIV-infected volunteers, compared to uninfected controls. Activation and immunosenescence of T cells—which occurs naturally during aging—were both associated with vascular dysfunction.

The individuals analyzed from the SCOPE study confirmed many of these observations. Although the treated HIV-infected volunteers had higher levels of proliferating CD4<sup>+</sup> T cells (as measured by Ki67 expression), the frequency of these cells able to divide *ex vivo* was low, as compared to the frequency of these cells in uninfected controls. These proliferative defects are also considered a common trait of an aging immune system.

The SCOPE data also showed that HIV-infected individuals on HAART had fewer naive CD8<sup>+</sup> T cells, and more CD8<sup>+</sup> CD28<sup>-</sup> T cells than age-matched HIV uninfected individuals, as well as elevated serum levels of C-reactive protein, a biomarker for aging that is usually triggered by infection or tissue injury.

Deeks also presented data from a recently published SCOPE-related analysis showing that among long-term ARV-treated individuals, the levels of cytomegalovirus (CMV)-specific T cells are dramatically expanded, with levels at least twice as high as that seen in age-matched uninfected individuals (*PLoS One* 5, e8886, 2010). CMV seropositivity is strongly associated with accelerated aging of the immune system. These cells have also been associated with heart disease in the HIV-infected population.

## Natural born killers

A number of presentations at Keystone also explored the role of innate immunity in preventing and controlling HIV infection. Galit Alter, assistant professor of medicine at the Ragon Institute, presented data from a study showing the first example of an innate immune-driven escape mutation in HIV-infected individuals, which could help lead researchers to a better understanding of how NK cells—thought to be non-specific—are able to recognize HIV. This data could also help researchers ascertain how HIV can evade the early expansion of certain NK cells.

Certain polymorphisms of the killer cell immunoglobulin-like receptors (KIRs) present on the surface of NK cells (including KIR3DS1 and some alleles of KIR3DL1), in combination with specific HLA-B alleles such as HLA-B57, are known to delay

progression to AIDS in HIV-infected people (see *Perspective: Natural Killer Cells: Bridging Innate and Adaptive Immunity?*, IAVI Report, May-June 2006). However, the mechanism for this is unclear.

Earlier experiments in acutely infected HIV-infected individuals have shown a remarkable expansion of NK cells following acute infection, with a preferential increase in the frequency of KIR-3DS1 in the presence of its putative ligand HLA-B Bw4801. Researchers have also found that certain subtypes of KIR-3DL1, a highly polymorphic allele, lead to different expression levels of this receptor on the surface of NK cells, and that the alleles that are expressed at high levels are also able to provide protection from disease progression in the presence of their HLA-B ligands.

To determine whether KIR on NK cells could place direct pressure on the virus *in vivo*, Alter, along with colleagues at the Ragon Institute and Microsoft Research, sequenced the viral DNA of 91 chronically infected individuals who were not on ARV therapy and looked for potential escape mutations associated with all known KIR receptors. They found 22 KIR-driven mutations and determined that at least two of these mutations directly provide a means by which the virus is able to avert detection by KIR-expressing NK cells.

Todd Allen at the Ragon Institute is now working with colleagues at the Broad Institute, a joint project between Harvard University and the Massachusetts Institute of Technology, to use ultra-deep sequencing to get a more in-depth look at viral evolution in larger sets of samples, and to expand this dataset to define all KIR-associated escape mutations that may occur in the virus to escape recognition by NK cells beginning in acute HIV infection. These studies will allow researchers to nail down exactly which variants of the virus select for the escape mutations and when the mutations occurred. In doing so, researchers hope to have a better idea of the role that innate immunity plays on viral containment and diversification.

Alter's group believes HIV may be evading recognition/detection by NK cells by interfering with the activating receptor NKG2D, which modulates NK-cell function, and the protein ligands MICA and MICA/B, which NKG2D expresses during periods of cell stress. Chronically infected, untreated individuals appear to secrete higher levels of MICA, which appears to dampen the expression of NKG2D on the surface of NK cells. Similar mechanisms of NK cell evasion have been reported in certain cancer models. Alter postulates that HIV

may be inducing these ligands, setting off a chain reaction that cleaves NKG2D and impairs NKG2D-dependent NK-cell mediated cytotoxicity. "Bottom line is, I think NK cells are playing an important role, early on, in HIV infection," said Alter.

### Visualizing transinfection

NK cells are not the sole component of innate immunity that has been occupying the minds of HIV researchers lately. Researchers are also focusing on dendritic cells (DCs).

DCs engulf and degrade HIV to present viral antigens to CD4<sup>+</sup> T cells. But in doing so, a portion of the virus can remain intact inside DCs. Two years ago, David McDonald, an assistant professor of cell and molecular virology at Case Western Reserve University, used antibody staining to show that these intact particles left inside DCs reside in compartments contiguous to the cell membrane, making it possible for the viral particles to be passed along to CD4<sup>+</sup> T cells in a process known as *transinfection* (*PLoS Pathog.* 4, e1000134, 2008; see Figure 3). This finding helped spur further study of how innate immune cells can both help and hinder the spread of HIV.

At Keystone, McDonald used imaging technology to show that cell-surface markers on the plasma membranes of the T cell entered the HIV pocket of the DC at the infectious synapse, a region on the surface of T cells where CD4 and its co-receptors are recruited.

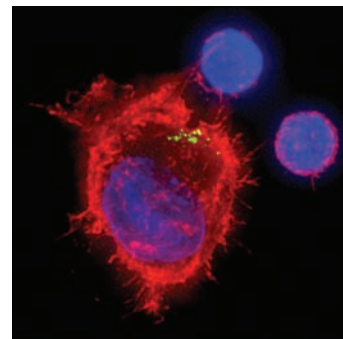
McDonald said there are ligands between T cells and DCs that enable the cell membranes to stick together. And when the T cell starts sniffing around looking for peptides presented by the DCs, the T cell also runs the risk of sticking its membrane into the intact virus. If that happens to be CD4 present on the surface of the T cell, HIV will slide in and infect it.

Although immune activation drives mucosal DCs into lymphoid tissues, where the vast majority of HIV replication occurs, it is not clear how important or extensive these interactions are. McDonald's group has hypothesized that DCs amplify HIV infection within lymphoid tissues by continually picking up and passing on infectious HIV during interactions with CD4<sup>+</sup> T cells. "Our hypothesis, through indirect evidence, is that DCs help to drive the disease," said McDonald. "But we don't have any *in vivo* evidence to support that. Our next push is to extend our live-cell imaging capability to study these cellular interactions within lymphoid tissues." ■

FIGURE 3

#### Viral Crossing

Image shows an infectious synapse where the larger human antigen-presenting cell is passing HIV virions (green) to the two smaller target CD4<sup>+</sup> T cells.



Courtesy of David McDonald, Case Western Reserve University

# *Antibodies:* Beyond Neutralization

In the search for correlates of protection, AIDS vaccine researchers are once again starting to look beyond the classic neutralizing antibody responses

By Andreas von Bubnoff

The types of immune responses that are generally considered important for vaccine-induced protection against HIV are T cells and neutralizing antibody responses. But when it comes to antibodies, there are other mechanisms that may play a role in vaccine protection. Antibodies that coat an HIV-infected cell can recruit innate immune cells to either kill the HIV-infected cell or otherwise inhibit viral replication. The killing of the HIV-infected cell is referred to as antibody-dependent cellular cytotoxicity (ADCC). The inhibition of viral replication as a result of the death of the HIV-infected cell or other mechanisms is referred to as antibody-dependent cell-mediated virus inhibition (ADCVI).

Recently, there has been speculation that ADCC could in part explain the results of RV144, an efficacy trial in Thailand of a prime-boost vaccine regimen that reduced the risk of infection by about 31% (*N. Engl. J. Med.* 361, 2209, 2009). In addition, ADCC appears to be higher in elite controllers—HIV-infected individuals whose plasma HIV RNA levels remain very low for a prolonged period of time without antiretroviral therapy. Studies in recent years also suggest that ADCC/ADCVI might play a role in the protection of rhesus macaques from challenge with simian immunodeficiency virus (SIV) or SHIV, an SIV/HIV hybrid.

“One of the ideas is that neutralizing antibodies maybe on their own are not sufficient,” says Galit Alter, an assistant professor of medicine at the Ragon Institute in Boston who studies ADCC/ADCVI. “But if they could recruit innate immune function, now all of a sudden maybe you are able to really confer protection against infection.”

## Initial neglect

After initial interest in the late 1980s and early 1990s, AIDS vaccine researchers have generally neglected studying ADCC/ADCVI until recently, Alter says. Instead, they focused their attention largely on eliciting T-cell immunity and neutralizing-antibody responses. But studies conducted about 20 years ago showed that ADCC-related mechanisms were a possible correlate of protection against HIV infection. In 1987, Kent Weinhold, a professor of surgery at Duke University, discovered ADCC in HIV-infected patients (*Proc. Natl. Acad. Sci.* 84, 4601, 1987). He found that CD4<sup>+</sup> T-cell lines with gp120 bound to their CD4 receptors were sensitive to lysis by peripheral blood mononuclear cells (PBMCs) from HIV-infected individuals, but not from uninfected people. He later found that PBMCs from HIV-infected individuals contained natural killer (NK) cells with anti-gp120 antibodies



bound to their Fc receptor, and it was these antibodies in HIV-infected individuals that recruited ADCC effector cells to the gp120 on the CD4<sup>+</sup> T-cell lines. “We were quite excited about the potential [of ADCC directing antibodies] as a correlate of immune protection,” Weinhold says.

An important aspect of this mechanism, according to Weinhold, is that it does not require major histocompatibility complex (MHC) class I receptors. Therefore, innate immune cells, in concert with anti-HIV antibodies, can mount an ADCC response against infected target cells that are from a different host, he says. This is different from T cells, which only get activated once their T-cell receptor recognizes that an infected target cell comes from the host. This may make it possible for ADCC to defend against early transmission with HIV-infected cells from an infected partner.

But several years later, Weinhold was involved in an experiment that turned him away from studying ADCC further. In the experiment, chimpanzees infused with a high titer of antibodies from HIV-infected people were not protected against an HIV challenge. “That was what turned us away from a focus on ADCC,” Weinhold says. “We thought it was an intriguing phenomenon and still do, but the inability to demonstrate any kind of protective effect kind of took us away from ADCC.” What’s more, he says, no one had really demonstrated that ADCC-directing antibodies were a correlate of protection in any animal model of human disease. “There wasn’t a precedent,” Weinhold says. “It was hard to write grants on any of this stuff because it was speculation. We didn’t have good evidence that it could serve as a correlate.”

At the same time, the knowledge that T cells and neutralizing antibodies are the correlate of immunity for other vaccine models has led many researchers to largely neglect ADCC over the past 20 years, Alter says. “Everybody jumped on the bandwagon and left ADCC in the background,” she says. “It’s the irony of the cyclical patterns of fashion in HIV research. Something becomes fashionable [and] everyone starts working on it. It’s just so funny how people follow the crowd.” It also didn’t help, she says, that it was a difficult functional response to look at (See *Detecting Antibody Activity*, page 10).

## Results from other fields

Meanwhile, developments in the fields of cancer research and autoimmunity also suggested that antigen binding and neutralizing activity of

an antibody via its Fab region (the tips of the Y-shaped antibody) is not the only thing that accounts for its biological activity. The Fc region (the base portion of the Y-shaped antibody) is also important, says Jeff Ravetch, a professor at Rockefeller University who has been studying Fc receptors for the past two decades. Ravetch found that in mouse models of autoimmune disease, development of the disease phenotype required the activation of a certain type of Fc receptor. He also showed that in mice, the activity of monoclonal therapeutic antibodies developed to treat certain cancers required Fc receptor activation and therefore ADCC. “ADCC was required for the *in vivo* activity of these antibodies,” Ravetch says.

Later, several clinical studies found that people with alleles of Fc receptors that bind better to the Fc regions of antitumor antibodies responded better to the antibody treatment. “So our prediction is that if you take an Fc domain and modify it so it binds more robustly to activating receptors and enhances ADCC, it will be a more effective therapeutic,” Ravetch says.

Drug companies have already taken notice. “The therapeutic antibody field has flipped over—now every therapeutic antibody is ADCC,” says Ravetch. “All the therapeutic drug companies are generating Fcs for enhanced ADCC activity, and clinical trials [have] now progressed to Phase III for Fc-engineered antibodies to enhance ADCC *in vivo*.”

**It’s the irony of the cyclical patterns of fashion in HIV research. Something becomes fashionable [and] everyone starts working on it.**

– Galit Alter

HIV vaccine researchers have more recently found additional hints to suggest that ADCC, ADCVI, or related mechanisms might be at play in protection from HIV infection. Don Forthal, an associate professor of medicine at the University of California in Irvine, found that polymorphisms in Fc receptors appear to track with HIV disease progression, possibly by changing the binding affinity of antibodies to Fc receptors. He found that people show faster disease progression if they have a variant of the Fc receptor gamma R2A that is less efficient at binding anti-

body (*J. Immunol.* 179, 7916, 2007). As a result, it is possible that more immune complexes stay in circulation, which could contribute to immune activation and accelerate the person's progression toward disease. "That got people excited," says Alter.

In addition, studies have shown that ADCC might play a role in protection from challenge of rhesus macaques with SIV and SHIV. Marjorie Robert-Guroff, head of the immune biology of retroviral infection section at the US National Cancer Institute, showed in several studies starting in 2005 that ADCC might account for at least some of the protection against SHIV and SIV challenge in macaques.

In 2007, Ann Hessell, a staff scientist in Dennis Burton's group at The Scripps Research Institute, and colleagues mutated the Fc receptor binding region of the broadly neutralizing antibody b12, knocking out its ability to bind to Fc receptors. As a result, four out of nine macaques infused with the mutated antibody became infected after high-dose vaginal SHIV challenge, while most (eight out of nine) macaques infused with wild type b12 antibody were protected (*Nature* 449, 101, 2007). "[The study] caused people to think that maybe we kind of gave up on ADCC or on these mechanisms a little bit too

early," Weinhold says.

The b12 mutant in the 2007 study eliminated interactions between b12 and all Fc receptor types, but Hessell and colleagues are making additional b12 mutants to see which of the effector cells recruited by certain types of Fc receptors are the most important. "We have now cloned a new panel of b12 Fc variants and are in the early stages of characterizing their phenotypes for effector function activities," Hessell says. Alter, who collaborates with Burton in these studies, also says it will be important to determine which type of innate immune cell is most relevant for ADCC/ADCVI protection. "If you knock out the ability of an antibody to bind to different innate immune components, you can start to tease out the effect of Fc receptors on protection from disease acquisition, which is huge," she adds. "We are trying to figure out, do you want to recruit NK cells, neutrophils, monocytes, dendritic cells? [And] which Fc receptors do you want to target?"

In addition, Hessell says that other broadly neutralizing antibodies like 2G12 might also protect at least in part by mechanisms other than neutralization, especially since a recent study found that 2G12 protects much better *in vivo* than would be expected from its neutralization

## Detecting Antibody Activity

### Antibody-dependent cellular cytotoxicity (ADCC) assays:

ADCC assays have three components: Target cells that express HIV antigens (CD4<sup>+</sup> T cell lines or primary CD4<sup>+</sup> T cells), effector cells such as natural killer (NK) cells, and a source of antibody such as serum, plasma, or monoclonal antibodies. ADCC assays measure the death of target cells by NK cells in the presence of antibody. This is usually done by measuring the release of a dye or another compound that the cells release once they die.

As target cells, researchers often use CD4<sup>+</sup> T-cell lines that are resistant to lysis by NK cells in the absence of antibody. The target cells are either infected with HIV or coated with gp120 or Env glycoprotein, and effector cells are added. One source for NK effector cells are peripheral blood mononuclear cells (PBMCs) from uninfected donors. These can be enriched with NK cells, so that the killing can then be assumed to be mostly due to NK cells.

Recently, Michael Alpert, from the lab of David Evans at Harvard Medical School, reported at the 27th Annual Symposium on Nonhuman Primate Models for AIDS that he is working on a standardized ADCC assay that provides less variable results because it uses cell lines for both the target and effector cells (see *Monkey Models: Far from Extinct*, *IAVI Report*, Nov.-Dec. 2009).

### Antibody dependent cell-mediated virus inhibition (ADCVI) assays:

The ADCVI assay also has three components: Infected target cells (primary CD4<sup>+</sup> T cells or CD4<sup>+</sup> T-cell lines), effector cells such as NK or other innate immune cells, and an antibody source. Unlike the ADCC assay, the ADCVI assay does not measure the death of infected target cells, but rather the degree to which NK cells or other innate immune cells used in the assay inhibit virus yield from the infected target cells in the presence of antibody.

Since this inhibition of virus replication can come from different kinds of innate immune cells, the effector cells used in ADCVI assays can be NK cells, unfractionated PBMCs, or other kinds of innate immune cells, such as monocytes or macrophages. The target cells in this assay can be primary CD4<sup>+</sup> T cells or a CD4<sup>+</sup> T-cell line that is resistant to lysis by NK cells in the absence of antibody.

The assay is usually done by infecting target cells with HIV for 48 hours. Then cell-free virus is washed off and the HIV-specific antibody and the effector cells of choice are added. About a week later, the virus is measured in the supernatant, usually by measuring the amount of HIV core protein p24. This is then compared with a setup where everything is the same except that the antibody is not against HIV. —*AvB*

ability *in vitro* (see 2G12 Revisited, *Research Briefs, IAVI Report*, July-August 2009). “There have got to be other things going on and of course we want to explore that,” says Hessel.

### Sufficient for protection?

Hessel thinks it is unlikely that non-neutralizing antibodies alone could be sufficient for protection via processes like ADCC. She says it is better to call antibody functions such as ADCC “extra-neutralizing” rather than “non-neutralizing.” “Both neutralizing and possibly non-neutralizing antibodies may contribute to ADCC,” she says.

But others say ADCC/ADCVI could have a possible protective effect even in the absence of neutralizing antibodies. ADCVI probably requires a lower binding affinity between the Fab part of the antibody and its cognate antigen than what is required for neutralization, Forthal says. “For an antibody to mediate ADCVI, all it has to do is attach to an infected cell with perhaps not the greatest affinity, but just enough affinity to hang on long enough to have a natural killer cell come by and allow crosslinking of the natural killer cell’s Fc receptors,” he says, adding that the same would be true for other innate immune cells that also have Fc receptors and can mediate ADCVI such as monocytes, macrophages, dendritic cells, or neutrophils. “So I think the threshold for this activity with respect to antibody affinity is probably lower, and in that sense there may be epitopes which allow ADCVI to occur that don’t allow neutralization to occur.”

Forthal says ADCC/ADCVI-related mechanisms could also explain why the prime-boost regimen tested in RV144 showed some efficacy even though the candidates do not appear to induce neutralizing antibodies. The vaccine regimen tested in the RV144 trial didn’t result in neutralizing antibody responses to primary HIV isolates, according to Josephine Cox, a director of clinical immunology at IAVI, who participated in that study (*J. Infect. Dis.*, 190, 702, 2004). Cox was also involved in a 2005 study that showed that the vaccine regimen did induce elevated ADCC activity (*Vaccine* 23, 2522, 2005).

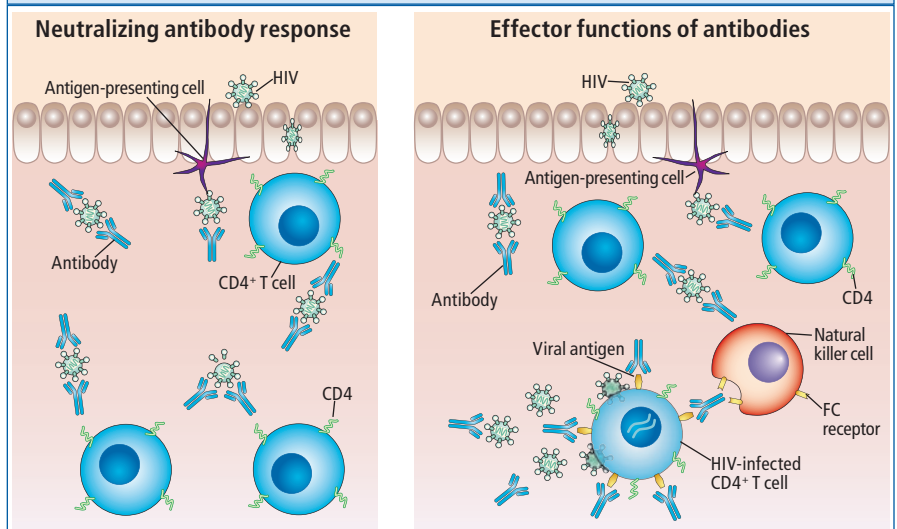
In addition, Forthal was part of a 2007 study that looked at ADCVI responses to antibodies from vaccinees of the Vax 004 Phase III trial of AIDSVAX B/B, which is similar to the gp120 AIDSVAX B/E boost used in RV144. While that vaccine candidate did not show any efficacy and

did not induce much of a neutralizing antibody response against clinical HIV strains, the study found that the lower the ADCVI activity, the higher the rate of HIV infection was after vaccination (*J. Immunol.* 178, 6596, 2007). “I know there was no overall efficacy in that [Vax 004] trial, but it may be that certain subgroups who have high levels of [ADCVI] antibodies were protected,” Forthal says. “We don’t expect a lot of classical neutralization by the antibodies of clinical strains of virus after that [RV144] vaccination, [but] it may very well be that ADCVI, ADCC, or some other Fc-receptor mediated non-neutralizing antibody may play an important role in this case.” As a result, there are discussions about measuring ADCC or ADCVI responses in blood samples from RV144, Forthal says.

Cox says she would also expect ADCC to be present in the mucosa. But measuring that in the RV144 trial will be difficult because very few

## Antibody Activities in Mucosal Tissues

In antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cell-mediated viral inhibition (ADCVI), the Fc regions of antibodies bound to HIV-infected cells bind to Fc receptors of innate immune cells such as natural killer (NK) cells, monocytes, or macrophages. Depending on the type of innate immune cell, and the Fc receptor involved, this binding either activates or inhibits the innate immune cell. Some innate immune cells such as NK cells then kill the target cell that has the antibody bound to it, as happens in the case of ADCC. This killing of target cells can be measured as ADCC in *in vitro* assays. In the case of ADCVI, the innate immune cell, upon Fc receptor binding, can secrete chemokines that inhibit viral replication, says Don Forthal, an associate professor of medicine at the University of California at Irvine, who developed an assay that measures ADCVI of HIV-infected cells (*J. Virol.* 75, 6953, 2001). Figure adapted by permission from Macmillan Publishers Ltd: *Nature* 449, 29-30, 2007.



mucosal samples were collected in the trial. “Unless we do further trials we are not going to be able to show that,” she says.

One challenge is that there is so far no standardized assay to measure ADCC or ADCVI, Weinhold says. Alter agrees. “We have no idea if all the ADCC assays are comparable or even what they are measuring,” she says. David Montefiori at Duke University Medical Center is organizing efforts to standardize the protocols and make the assays comparable. “We are indeed organizing an effort to examine the readiness of various ADCC and ADCVI assays for case control samples from the RV144 trial,” Montefiori says. “Our goals are to evaluate intra-laboratory variability and to compare results between assays.” There are also efforts to develop new assays with better defined components (see *Monkey Models: Far from Extinct, IAVI Report*, Nov.-Dec. 2009). Ravetch says the RV144 trial volunteers should also be analyzed for the types of Fc receptor alleles they have, which could affect their ADCC or ADCVI response.

## Both neutralizing and possibly non-neutralizing antibodies may contribute to ADCC.

— Ann Hessell

But not everyone believes there is enough evidence to suggest that ADCC or ADCVI may have a role in the small efficacy observed in RV144. “I think there’s no meaningful evidence that ADCC or ADCVI is either protective against HIV transmission or involved in controlling HIV infection *in vivo*,” says John Moore, a professor of microbiology and immunology at Weill Cornell Medical College, adding that he doesn’t trust the relevance of many of the assays that have been used to measure these responses. He says the only paper on this topic that is of any real interest is the 2007 study by Ann Hessell and colleagues on the effect of Fc-mutated monoclonal antibodies in passive protection studies (*Nature* 449, 101, 2007). “But it’s a big leap of faith to extrapolate from that work to the RV144 trial and whatever effect those vaccine components may, or may not, have had on acquisition,” Moore says.

### Elite controllers

Researchers are also investigating whether ADCC-like mechanisms might account in part for the viral load control seen in elite controllers. One recent study found that ADCC was higher

in elite controllers (*AIDS* 23, 897, 2009).

In a separate project, Alter is collaborating with William Hancock at Northeastern University to see if anti-HIV antibodies in elite controllers differ from antibodies in chronic progressors, for example in the structure of the glycans in their Fc receptor binding region, which modulate the binding affinity to Fc receptors of effector cells. Differences in the strength of Fc receptor binding to IgG antibodies have already been shown to explain part of the differences in the efficacy of therapeutic antibodies used to treat cancer, Ravetch says, and might explain the higher degree of ADCC seen in elite controllers. With Hancock and Genoveffa Franchini at the National Cancer Institute, Alter is also planning to look at whether ADCC could account for the protection some vaccine candidates have afforded in nonhuman primate studies.

Eventually, such studies might result in the identification of markers on antibodies that researchers might want to induce when they develop vaccine candidates. “There may be a chance that we can improve the protective nature of a vaccine if it can induce antibodies that can recruit this function,” Alter says.

For too long, the AIDS vaccine field has focused on the antigen binding region of antibodies and neglected the Fc region, which is involved in ADCC and ADCVI, Alter says. “I think that on antibodies we are missing the boat,” she says. “[People] are not even thinking about the larger section of the antibody which is the constant region.”

Ravetch also urges the AIDS vaccine field to take a closer look at Fc receptor-mediated mechanisms. “Stop being so narrow minded and thinking about everything the same way,” he says.

Not everyone is convinced. “Overall, I think ADCC/ADCVI is just another of the bandwagons that roll along every now and then in the HIV vaccine field,” says Moore. “Some people will be interested in joining it for a while, but not me.”

But others say that views in the field may already be changing. “I think everyone is sort of starting to believe [ADCC] is important—it’s just not sure how important it is because there is not a lot known on this subject,” Alter says. Weinhold agrees. “I think with Burton’s [2007] paper and now the speculation that accompanies the 31% efficacy in the Thai trial, people are now putting [ADCC] back on the table,” he says. “If there is any inkling or hint of ADCC as a protective mechanism in the Thai trial, I think it will spur people to revisit this with some of the technologies that we have now that we didn’t have 20 years ago.” ■

# PREVENT *and Conquer*

A collection of key research updates that headlined this year's Conference on Retroviruses and Opportunistic Infections

By Kristen Jill Kresge and Richard Jefferys

THE SUCCESS OF ANTIRETROVIRAL (ARV) treatment is a remarkable victory in the now 29-year-old battle against HIV. In his plenary talk at the 17th Conference on Retroviruses and Opportunistic Infections (CROI), which was held from February 16-19 in San Francisco, Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID), called ARV therapy “one of the best success stories in biomedical research as it gets translated to the patient.”

At the end of 2008, approximately four million HIV-infected people in low- and middle-income countries were receiving ARVs. “As the years go by we’re doing better in getting drugs to people who need them,” said Fauci. “That’s the good news.”

“The sobering news,” Fauci continued, “is that it’s not sustainable.” Despite this progress, there is still a huge gap between the number of people who need treatment and those who receive it—a gap recently widened by the World Health Organization’s (WHO) decision to revise treatment guidelines in response to mounting evidence for the benefits of earlier initiation of ARV therapy (see *Everything from Cause to Cure, IAVI Report* July-Aug. 2009). Based on the updated guidelines, only 30% of the HIV-infected people in the world who qualify for therapy are receiving it.

Closing this gap is a huge priority in battling

HIV/AIDS, and critical to that is reducing the number of new infections. Fauci outlined a triumvirate of HIV prevention strategies that top the research agenda at NIAID, including the development of a preventive HIV vaccine; test and treat, which calls for universal HIV testing and immediate treatment for those infected; and pre-exposure prophylaxis, which involves ARVs delivered orally or in a microbicide gel to uninfected individuals. Several research updates on these three areas dominated the discussions at this year’s CROI. Another priority at NIAID is finding a cure for HIV infection. Fauci said all of these efforts relate to a common goal, which is “controlling and ultimately ending the HIV/AIDS pandemic.”

## Building on RV144

One of the main planks in the HIV vaccine research agenda at NIAID is building on the results of the Thai trial, which showed that a prime-boost regimen (a canarypox vector prime followed by an HIV protein boost) provided about 31% protection against HIV infection (*N. Engl. J. Med.* 361, 2209, 2009). Building on these results will involve further clinical research, as well as more basic discovery research to support a better understanding of vaccine design and development.

## [ UPDATE ON RV144 ]

Nelson Michael, director of the US Military HIV Research Program, opened the session on HIV vaccines at the 17th Conference on Retroviruses and Opportunistic Infections by summarizing the results of RV144, the first AIDS vaccine trial to show any efficacy (see *Raft of Results Energizes Researchers, IAVI Report, Sep.-Oct. 2009; N. Engl. J. Med.* **361**, 2209, 2009).

Subsequently, investigators have conducted a post-hoc evaluation of risk behaviors of the trial participants, as reported at six-month intervals. Before sharing the results, Michael issued this disclaimer: “Your eyebrows should raise anytime any trialist talks about a post-hoc analysis.” He then showed that having reported high-risk behavior at any time during the trial, significantly impacted vaccine-induced protection (p value of 0.008). Michael suggested the association of risk with lack of protection may have more to do with the seemingly transient impact of vaccination—the protective effect of vaccination appears to have been concentrated almost entirely during the first 12 months of the three-year trial—than with risk, since the reporting of risk behaviors continued throughout the trial.

Michael also presented data on the titers of binding antibody against gp120 among the vaccinated volunteers. Comparing the magnitude of these responses at two and 24 weeks after the final immunization, Michael demonstrated that they dropped precipitously; the average geometric mean titer of binding antibody against the recombinant CRF01\_AE gp120 was 14,558 at the two-week time point and 1,000 at 24 weeks. —RJ

With regard to clinical development, Fauci said the RV144 results may have shifted the focus. “We really have to focus future trials on the prevention of acquisition,” said Fauci. “Understanding the T-cell response [through trials of candidates that are solely designed to impact viral load] is very important, but when we do a large clinical trial in humans, it is my opinion that we’ve really got to look at acquisition.” Fauci also set a high bar for the target efficacy for an HIV vaccine. “I think we’ve got to do better than 60%-70%,” he said, suggesting dramatic improvements on the Thai trial results would be necessary. “We’re setting the bar very high, but the history of AIDS tells us we’ll clear that bar with the best minds, resources, and political will.”

One obvious way to improve upon the RV144 results is to try to determine the immune correlates of protection. Nelson Michael, director of the US Military HIV Research Program (MHRP), who provided an update on some of the post-hoc analyses of RV144 (see *Update on RV144*, at left), said work on trying to determine the correlates of protection is just getting underway, with results expected in a year. Due to the limited number of samples available and the overall low HIV incidence rates during the trial, determining the correlates will likely be difficult. “We may not know what the correlates of protection are,” said Fauci, “but we will know, and this is really important, what they’re not.”

Many researchers have all but ruled out broadly neutralizing antibodies as the correlate of protection in RV144, since neither candidate induced such responses in previous trials. “[The Thai trial] tells us you can prevent acquisition without traditional neutralizing antibodies,” said Fauci.

If correlates of protection can’t be identified from RV144, it is possible that additional efficacy trials will be conducted. And as Michael pointed out, this will require other funding organizations, such as NIAID and the Bill & Melinda Gates Foundation, to pitch in. “The Army could bring US\$8 million a year to any further study we would probably do,” said Michael, who noted that the most expensive year of the Thai trial cost \$17 million. “We can’t go rogue and do another study by ourselves.”

## Studies of neutralizing antibodies

Other HIV vaccine research efforts are focused on studying broadly neutralizing antibodies against HIV, with the ultimate goal of reverse engineering vaccine immunogens capable of inducing these antibodies. However, a provocative presentation by Julie Overbaugh, a member of the Human Biology

Division at the Fred Hutchinson Cancer Research Center, raised questions about whether neutralizing antibodies can actually block acquisition of HIV. “We really don’t know yet if antibodies provide some benefit in protecting against HIV in exposed populations,” said Overbaugh.

In nonhuman primates, studies have shown that passive administration of the broadly neutralizing antibody b12 can block infection with a simian immunodeficiency virus/HIV (SHIV) challenge. The b12-treated macaques eventually become infected, but only after substantially more challenges than control animals (*Nat. Med.* **15**, 951, 2009). However, the challenge virus used in these studies was the most neutralization sensitive to b12, according to Overbaugh. “These experimental models provide important proof of principle that these approaches can work, but they focus on optimal combinations of viral strain and neutralizing antibodies. It’s hard to extrapolate that such levels of antibodies with that specificity would neutralize most circulating strains of HIV. The real world is not so ideal,” she said.

To see whether neutralizing antibodies present at the time of exposure could protect against infection in the natural setting, Overbaugh and colleagues studied HIV-infected mothers who breast feed their infants. Overbaugh presented data from an unpublished study of 100 infants, who were HIV uninfected at birth, whose mothers had broad and potent neutralizing antibodies. The maternal antibodies were passively transferred to the infants and were present at birth and during the breast-feeding period. Serum samples from the infants showed that some had “very broad and potent neutralizing antibodies that were passed on from their mothers,” according to Overbaugh. But when she compared the neutralizing antibodies in the 32 infants who acquired HIV during the period of breast feeding to those who remained uninfected, she found there was no difference in their breadth or potency. “In the setting of mother-to-infant transmission there’s no evidence that a broad and potent neutralizing antibody response as measured against a heterologous panel of viruses provided protection from HIV infection,” she said.

Overbaugh said the results might be different with the broadly neutralizing antibodies like PG9 and PG16, which were described in 2009, since they are able to neutralize many more viruses. Fauci said this is why passive immunization studies with some of the newer broadly neutralizing antibodies should be conducted.

## Evolution of neutralizing antibodies

Previous research on the evolution of neutralizing antibodies in HIV-infected people has shown that although these antibodies emerge several months after HIV infection, they are unable to neutralize contemporaneous HIV isolates from the same individual (*Proc. Natl. Acad. Sci.* **102**, 18514, 2005). Frederick Hecht from the University of California in San Francisco, who presented data from a study addressing the long-term evolution of antibody responses to the shape-shifting HIV envelope in people with HIV infection, found that instead, the antibodies seem to be playing a constant game of catch-up—they are only capable of neutralizing autologous viruses sampled three to four months previously.

Hecht showed that this phenomenon continued for close to four years in the majority of the 13 study participants, with antibody responses continuing to evolve, but always lagging behind the virus.

But when Hecht analyzed the neutralizing activity of these antibodies from the last sample collected in the four-year study, he found that all 13 participants possessed antibodies capable of significantly inhibiting a panel of 12 heterologous HIV isolates. Depending on the concentration cut-off, an average of 50-87% of the panel could be neutralized. Hecht and colleagues hypothesized that the generation of new waves of neutralizing antibodies against autologous virus would correlate with the broadening of activity against the heterologous virus panel. In a multivariate model including CD4 count, viral load, duration of infection, and the cumulative evolution of autologous neutralizing antibodies, this indeed proved to be the case—only the latter factor significantly predicted the breadth of heterologous neutralization, with a p value of <0.0001.

The rationale for this study was partly derived from a study by Hecht's colleague Larry Bragg, who presented a poster at CROI (see *Superinfection Risk May Decline Over Time*, at right) indicating that one year after acquisition of HIV infection, the risk of superinfection drops significantly. Hecht concluded by suggesting that the time course of antibody evolution documented in the study was consistent with the data on decreased risk of superinfection, and that this may represent a model of protection with important implications for vaccine design.

## Locking onto lipids

In 2001 it was reported that persistent infection with GB virus type C (GBV-C) was associated with prolonged survival in people with HIV (*N. Engl. J.*

*Med.* **345**, 715, 2001). GBV-C is a flavivirus that is not known to cause disease, and the mechanism by which it might impact HIV infection has remained shrouded in mystery. At CROI, Heide Reil, from the University Hospital in Erlangen, Germany, presented data showing that, surprisingly, some antibodies directed against the E2 glycoprotein of GBV-C can neutralize a wide range of different HIV isolates even though there is no homology between the two viruses. Reil's investigation was prompted by data in the 2001 *NEJM* paper suggesting that people with HIV who have cleared GBV-C and possess anti-E2 antibodies still show attenuated disease progression, even though the magnitude of the effect was reduced compared to individuals who have persistently detectable GBV-C viremia.

Reil described initial experiments conducted with a polyclonal mix of different monoclonal antibodies directed against E2, which revealed broad neutralization of a panel of HIV isolates from multiple clades, including C and D, which are typically hard to neutralize. Further analyses showed that just two of the monoclonal anti-E2 antibodies, dubbed M6 and M11, could recapitulate this activity on their own. However, neither could neutralize other viruses tested, including murine moloney virus, yellow fever, and adenovirus serotypes 5 and 12.

Intrigued by these observations, Reil and colleagues embarked on an iterative series of studies that revealed the neutralizing activity was not directed against HIV envelope glycoproteins, but rather phospholipids contained in the viral membrane. This class of lipids inhabits the inner layer of cell walls and they are acquired by HIV particles as they bud. Reil noted that two broadly neutralizing monoclonal antibodies against HIV, 2F5 and 4E10, are also known to bind phospholipids. In those cases, a mechanism has recently been suggested which involves a two-step process: the antibodies initially bind phospholipids on the viral membrane, which then allows them to be in close proximity when the epitope they target in HIV envelope's membrane proximal region (MPER) is very briefly exposed during fusion of the virus to the target CD4 cell (see Figure 4, page 16). This recently published mechanism was described at CROI in a separate talk by Bing Chen (*Proc. Natl. Acad. Sci.* **106**, 20237, 2009).

To assess whether the M6 and M11 antibodies were targeting the same phospholipids, Reil tested their binding to an array of different types, finding that they bound a phosphatidylinositolphosphate called PI(4)P<sub>2</sub> (see *Targeting Phospholipids*, at right). In contrast, 2F5 and 4E10 bound to car-

## [ SUPERINFECTION RISK MAY DECLINE OVER TIME ]

Larry Bragg, a research associate at the Gladstone Institute of Virology and Immunology at the University of California in San Francisco, presented a poster suggesting that among people recently infected with HIV, the risk of superinfection with a second virus strain declines significantly after one year. The data were obtained from the OPTIONS study and involved 256 individuals with recent HIV infection, nine of whom were found to have acquired a highly divergent second strain of HIV. Of these cases, six occurred in the first year, one in the second, and two in the third. Although the numbers are small, statistical analyses indicated an approximately 21-fold reduction in risk of superinfection after the first year (p value of 0.005). Bragg and colleagues suggested that biological mechanisms may account for the finding (see *Evolution of neutralizing antibodies*, this page), but also acknowledged that other explanations are possible, such as variations in individual susceptibility. —RJ

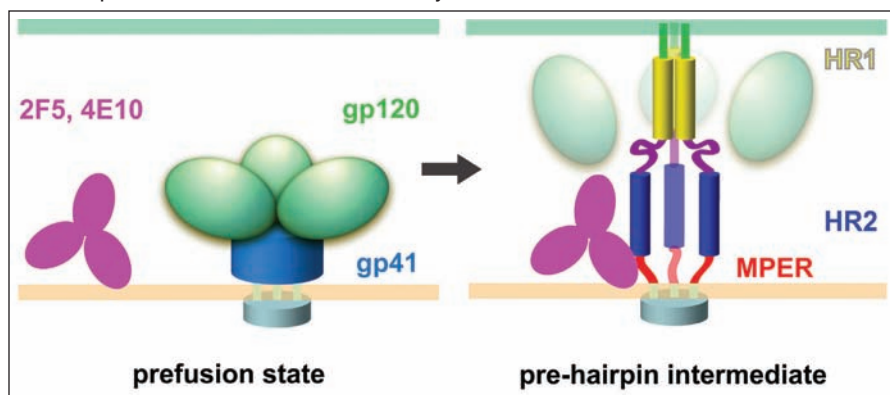
## [ TARGETING PHOSPHOLIPIDS ]

In a plenary talk at CROI, Hans-Georg Kräusslich, a professor at the University of Heidelberg, reported data suggesting that HIV specifically recruits phosphatidylinositolphosphate PI(4,5)P<sub>2</sub>, when assembling the viral membrane (see *Locking onto lipids*, this page). His group is now working to understand the process in more detail. Commenting on whether it might be possible to target HIV using this knowledge, Kräusslich said, "I don't know but there is at least some promising reason to study it." Targeting phospholipids may not carry the risk of autoimmunity typically associated with host cell components, because one of their functions is to promote non-inflammatory clearance of apoptotic cells (*Molecules* **14**, 4892, 2009). —RJ

diolipin, and cardiolipin and phosphatidylserine respectively, as has previously been reported (*Science* 308, 1906, 2005). Reil concluded her talk by suggesting that the E2 glycoprotein, and perhaps even entire GBV-C particles, should be considered as antigens for future HIV vaccine strategies.

FIGURE 4

A two-step model for neutralization of HIV-1 by MPER-directed antibodies (2F5 and 4E10).



Courtesy of Bing Chen; also published in *PNAS* 2009, 106:20237.

### Live-attenuated protection

For nearly two decades, researchers have been trying to unravel the mechanisms underlying the protection afforded by live-attenuated SIV vaccines in rhesus macaques. At CROI, Paul Johnson, chair of the division of immunology at the New England Primate Research Center, reviewed data from multiple challenge experiments using the attenuated vaccine SIVmac239 $\Delta$ nef, developed by Johnson's colleague Ron Desrosiers. These studies have consistently shown sterile protection against homologous SIV isolates administered intravenously. And although protection against a high-dose mucosal challenge with the heterologous SIVsmE660 has not been achieved, immunized animals display enhanced post-infection control of viremia. Johnson also cited a recent unpublished study conducted by Matt Reynolds involving repeated low-dose mucosal challenges that showed this live-attenuated vaccine resulted in a significant delay in time of acquisition of SIVsmE660, along with control of viral load in animals that became infected.

Johnson described several studies conducted to delineate the contribution of antibodies to the protection observed with SIV $\Delta$ nef. The first, by the research group of Jörn Schmitz at Beth Israel Deaconess Medical Center in Boston, administered the B cell-depleting monoclonal antibody rituximab to 10 vaccinated macaques for 100 days

prior to challenge with SIVmac239, leading to persistent depletion of B cells in five of the animals. Despite the absence of B cells and antibody responses, four of five were completely protected and the remaining animal has controlled viremia through 200 days post-challenge.

In another unpublished study summarized by Johnson, Ron Desrosiers employed a different approach that cleverly took advantage of the poorer protection SIV $\Delta$ nef offers against SIVsmE660. Desrosiers swapped the SIVmac239 envelope gene from SIV $\Delta$ nef with another derived from SIVE543, which is closely related to SIVsmE660. Immunogenicity analyses have uncovered no cross-neutralizing antibodies against these two envelopes. An experiment was then conducted in which 24 macaques were divided into two groups and received either SIV $\Delta$ nef or SIV $\Delta$ nef/EnvE543. An SIVmac239 challenge virus was also created with the envelope of SIVE543 and six animals in each group were challenged with wild type SIVmac239 and six with SIVmac239/EnvE543. This approach allowed each group to receive both homologous and heterologous challenges. No vaccinees in either group challenged with homologous virus were infected, and only two of 12 that received a heterologous challenge were infected. Considering the data from Desrosiers and Schmitz, Johnson concluded that antibody responses are unlikely to play a major role in the protection observed with live-attenuated vaccines.

Johnson next explained that ongoing antigenic stimulation by SIV $\Delta$ nef does appear to play a role in protection, citing data using a single-cycle SIV that, as its name suggests, can only complete one round of infection. Published studies using this construct as a vaccine have shown only around a log reduction in post-challenge viral load compared to the complete protection offered by live-attenuated SIV (*PLoS Pathog.* 5, e1000272, 2009).

In the last section of his talk, Johnson focused on the role of CD8<sup>+</sup> T-cell responses in explaining the efficacy of SIV $\Delta$ nef. To address this issue, his laboratory performed a study in which three groups of macaques were challenged intravaginally with SIVmac251 at different timepoints after immunization. Each group consisted of nine animals, six that received SIV $\Delta$ nef and three controls. The challenge was administered at 5, 20, or 40 weeks after vaccination. No protection was seen at five weeks, whereas three of six macaques challenged after 20 weeks were protected, as were two at week 40. All but one of the nine controls became infected.



A starker contrast emerged when he looked at post-challenge viremia in these animals. Although the magnitude of SIV-specific CD8<sup>+</sup> T-cell responses peaked at five weeks, the group challenged at this timepoint showed a log reduction in peak viral load compared to the controls. However, the difference between the two groups was extremely short-lived. In contrast, both the 20- and 40-week groups showed far greater reductions in viral load that remained more than two logs lower than controls through about 40 weeks.

A comprehensive analysis of SIV-specific CD8<sup>+</sup> T-cell responses revealed that expression of the central memory markers CCR7 and CD127 (also referred to as IL-7R, the receptor for IL-7) was significantly higher at 20 and 40 weeks. But Johnson cautioned against leaping to the conclusion that this CD8<sup>+</sup> T-cell phenotype was the cause of enhanced control of viral load, as similar expression levels of these molecules have been reported after single cycle SIV immunization. The marker that distinguished the two approaches was programmed death (PD)-1, once thought to signal T-cell exhaustion, but now known to also be upregulated on activated T cells, leading Johnson to suggest that in SIV $\Delta$ nef vaccinated animals its expression was serving as an indicator of persistent antigenic stimulation.

Johnson proposed what he called the Goldilocks principle: “You need just the right amount

of ongoing antigenic stimulation,” he said, “too much and all of your cells end up as effector memory cells, too little, they turn into central memory cells.” His opinion, at least in these models, is that SIV $\Delta$ nef seems to be just right.

### The test and treat approach

When researchers at the WHO published results from a mathematical model showing that universal annual testing and immediate ARV treatment for all HIV-infected individuals could make a major dent in the number of new HIV infections, the model ignited discussions among researchers and stimulated new research into the feasibility of the so-called test and treat approach (*Lancet* 373, 48, 2009). A NIAID-funded pilot study of test and treat in Washington, D.C. and New York City is scheduled to start in a few months (see *Vaccine Briefs, IAVI Report*, Sep.-Oct. 2009).

Despite the attention test and treat has received, there is limited data to support the premise. “All of the mathematical models assume much lower HIV transmission rates when on ARV therapy but there is very little empiric evidence,” said Deborah Donnell, deputy director of the HIV Prevention Trials Network (HPTN) statistical center.

This is beginning to change. At CROI, Donnell presented data that helps bolster the connection between ARV treatment and prevention. In an

## Researchers Still Grappling with Transmission

As early as 1992, researchers observed that a single HIV founder virus establishes infection in cases of heterosexual transmission. George Shaw, a professor at the University of Alabama at Birmingham, who delivered the Bernard Fields Memorial Lecture at this year’s 17th Conference on Retroviruses and Opportunistic Infections (CROI), has confirmed in much larger cohorts from acute HIV infection studies that a single founder virus establishes infection in 80% of heterosexual transmissions (see *HIV Transmission: The Genetic Bottleneck, IAVI Report*, Nov.-Dec. 2008).

But at CROI, Barbara Felber, chief of the human retrovirus pathogenesis section at the National Cancer Institute, presented data from a study in rhesus macaques that suggests researchers may be missing some of the transmitted viruses that only begin replicating at detectable levels in the chronic phase of infection. In this study, six rhesus macaques were infected either intra-rectally or intra-vaginally with SIVmac251, a swarm of virus variants with a maximum divergence of 2.3% in the entire Env region and 4% in the V1/V2 loops. The viral loads in the monkeys peaked at 7.5 log HIV RNA copies/ml of blood during the acute phase of infection (two weeks after infection) and 5.8 log RNA copies/ml in the chronic phase of infection (30 weeks post-infection).

Felber then sequenced the virus variants present at both stages of infection using single genome amplification. The power calculations employed were capable of identifying any genetic variants that represented at least 5% of the total virus population. In three of the six animals, Felber found that the genetic sequences present during acute infection fit into a “nice Poisson distribution,” suggesting they were infected by a single founder virus. The founder virus, however, was not the same, indicating that multiple variants in the challenge stock could establish infection. In chronic infection, Felber observed that two of these three animals had some virus variants that could not have evolved from the acute virus.

In two other animals, two virus variants were detected in the acute phase. Both animals also had additional variants present in the chronic phase.

In total, five of six animals had multiple variants in the chronic phase that were not detected during acute infection. Felber said that multiple founder viruses cross the mucosal barrier, and while only one or two of these replicate at detectable levels during the acute phase, other variants may emerge to prominence later, during the chronic phase. She suggested that only estimating the number of transmitted virus variants in humans during the acute phase may be inaccurate. —KJK

observational sub-study of the partners in prevention study—a study that showed treatment of herpes simplex virus-2 did not reduce risk of HIV infection—Donnell and colleagues analyzed HIV transmission rates among 3,381 serodiscordant couples from seven countries in eastern and southern Africa.

At the start of the study, the HIV-infected partners, the majority of whom were female (68%), had CD4<sup>+</sup> T-cell counts greater than 250 and were not already taking ARVs. CD4 levels in the infected partners were measured every six months. During the study, 10% (349) of the HIV-infected partners initiated ARV treatment. Half of those that started therapy had CD4<sup>+</sup> T-cell counts below 200 cells/microliter of blood.

At the end of the two-year study, 151 new HIV infections had occurred. Researchers confirmed, by viral sequencing, that 108 of the infections were linked to the HIV-infected partner in the study. Of the 103 infections included in the final analysis, only a single one occurred when the HIV-infected partner was on ARV treatment. This correlates to a statistically significant 92% reduction in HIV transmission if the infected partner was on ARVs. “There was a substantial prevention benefit for ARV therapy,” said Donnell.

Investigators observed that the infected partners who were not taking ARVs were much more likely to transmit HIV at lower CD4<sup>+</sup> T-cell counts. However, even at higher CD4<sup>+</sup> T-cell levels, there were still a higher number of HIV infections that occurred between couples if the infected partner was not on ARVs. “HIV transmission occurred across all CD4<sup>+</sup> T-cell levels,” Donnell said. Still, she stressed that the highest priority is getting individuals with the lowest T-cell counts on therapy.

An ongoing study, HPTN 052, is a randomized, five-year, Phase III clinical trial designed to determine HIV transmission rates among 1,750 serodiscordant couples in which the infected partner begins ARV therapy immediately, or only when their CD4<sup>+</sup> T-cell count falls between 200 and 250 cells/microliter. Researchers hope this study will provide a conclusive answer about the protective effects of ARV therapy.

Another method researchers are employing to gauge the ability of ARV treatment to reduce HIV transmission rates is estimating the community viral load—the mean viral load of all HIV-infected individuals in a given community. And in some cases, declines in community viral load are correlated with declines in the number of individuals newly diagnosed with HIV.

Moupali Das-Douglas, director of the research unit at the San Francisco Department of Public Health, presented data indicating that a significant 40% decrease in the community viral load among men who have sex with men in San Francisco that occurred between 2004 and 2008, correlated with a 45% reduction in the number of new HIV infections during this same four-year period. Additionally, from 2006-2008, Das-Douglas reported that the estimated HIV incidence, which reflects the number of new HIV infections both diagnosed and undiagnosed, also decreased by 33%; however, this was not a statistically significant reduction.

The declines in community viral load and the number of new HIV diagnoses were credited to an increase in HIV testing rates in San Francisco, as well as an increase in the number of infected individuals who are receiving ARVs. By 2008, 90% of HIV-infected individuals with AIDS in San Francisco were receiving ARV treatment. But although there is a statistically significant correlation, this study did not show directly that testing or more widespread ARV treatment was actually what caused the decline in the number of new HIV diagnoses. Still, Das-Douglas concluded that public health departments should consider measuring community viral loads. “What gets measured gets managed,” she said.

Julio Montaner, director of the British Columbia Centre for HIV/AIDS and a proponent of test and treat, reported similar results from a prospective study in British Columbia, Canada, which evaluated the community viral load of all HIV-infected people in the province who are on ARV treatment. Montaner said the rapid uptake of HAART in this population is “driving down viral load steadily,” and that this has resulted in a decrease in the number of new HIV diagnoses, particularly among injection-drug users (IDUs).

In 2004, approximately 50% of IDUs in British Columbia had viral loads greater than 1,500 copies of HIV RNA/ml of blood, compared to fewer than 20% in 2009. Even though the number of HIV tests administered in the study population has risen steadily, according to Montaner, there was a 50% decrease in the number of new HIV diagnoses among IDUs over this five-year period. He credited this reduction in new diagnoses among IDUs to the expansion of ARV therapy in this community, rather than any behavioral modifications. He says there is “plenty of room to improve” on these results, and he hopes to move ahead with what he calls the “seek, test,

treat, and retain” strategy with new support from the Canadian government. “We should proceed expeditiously.”

A third study conducted in Washington, D.C., which has the highest HIV prevalence in the US with about 3% of the population living with HIV/AIDS, showed a different trend than what was observed in San Francisco and British Columbia. Researchers from George Washington University School of Public Health and Health Services reported an increase in the number of new HIV diagnoses following a dramatic expansion of routine HIV testing services and efforts to provide those infected with treatment.

A routine HIV testing campaign began in Washington, D.C. in 2006. That year, approximately 35,000 HIV tests were conducted. By 2009, 93,000 HIV tests were performed. And the data suggests that efforts to link people with treatment were successful. In 2004, 23% of HIV-infected individuals in D.C. were entered into an HIV treatment and care program more than 12 months after receiving their HIV diagnosis. This number dropped to 5.4% in 2008. The median CD4+ T-cell count of HIV-infected individuals when they started taking ARVs also increased from 216 cells/microliter of blood in 2004 to 343 in 2008. Additionally, the percentage of people who progressed to AIDS within 12 months of their HIV diagnosis dropped from nearly 50% in 2004 to 28% in 2008.

Yet Amanda Castel, an assistant research professor at George Washington University, reported that from 2004 to 2007 there was a 17% increase in the number of HIV diagnoses in Washington, D.C., from approximately 1,100 to 1,300.

Kimberly Smith, an associate professor at Rush University Medical Center in Chicago, said that late diagnoses are partly responsible for the increased mortality from HIV within the black community in the US. “Black individuals are much more likely to die from HIV/AIDS than white individuals,” said Smith, which is why “initiatives like that in D.C. will be very important.”

## Intermittent PrEP

While randomized trials and feasibility studies of test and treat have only recently started, several clinical trials of PrEP will soon be yielding results. Kenneth Mayer, an investigator involved in a PrEP trial in the US, said 2010 will be a “major year in our understanding of PrEP.” The results of four oral PrEP trials and one trial of topical PrEP (a microbicide gel formulation of tenofovir) are expected this year.

Two new PrEP studies (HPTN 066 and 067) are also slated to begin this year. These trials are evaluating the intermittent use of PrEP, rather than daily. “Intermittent PrEP dosing may be a more feasible approach for many populations,” said J. Gerardo Garcia-Lerma, a researcher at the US Centers for Disease Control and Prevention. The preliminary results of a pilot feasibility study of intermittent PrEP conducted by IAVI are also expected this year. This study, in addition to HPTN 066 and 067, which will collect extensive pharmacokinetic data, will hopefully shed light on the optimal dosing regimens for intermittent PrEP.

At last year’s CROI, Garcia-Lerma presented results from an intermittent PrEP study in rhesus macaques that showed that when the drug Truvada (a combination of tenofovir and emtricitabine or FTC) was administered two hours before or after a low-dose rectal SHIV challenge, there was a four-fold reduction in risk of infection compared to untreated control animals (*Sci. Transl. Med.* 2, 14ra4, 2010). If Truvada was administered either one, three, or seven days prior to SHIV exposure with a post-exposure dose two hours after challenge, there was also a high level of protection, with a reduction in risk of infection ranging from 9- to 17-fold compared to untreated controls. “If the drug was administered close to the time of exposure, there was significant protection,” said Garcia-Lerma.

To better understand the relationship between this protection and the drug levels of Truvada, Garcia-Lerma and colleagues conducted single-dose pharmacokinetic studies in rhesus macaques. Drug concentrations in four animals were measured in plasma, peripheral blood mononuclear cells (PBMCs), and rectal secretions, and necropsy was used to collect lymphoid and rectal tissues from seven additional animals to determine tenofovir concentrations at these sites.

The results indicate that the concentration of the two drugs varies greatly between different sites. Garcia-Lerma said that both tenofovir and FTC persisted for a long time in PBMCs—the half-life of tenofovir diphosphate was about five days in these cells, similar to what is observed in humans, and the half-life for FTC was one day. In rectal secretions, however, the concentration of both drugs peaked at 24 hours. And, in plasma, drug levels of both tenofovir and FTC peaked after just two hours and then declined over one day. These findings suggest that the timing of intermittent PrEP may be important. “We have

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Intermittent PrEP dosing may be a more feasible approach for many populations.

– J. Gerardo Garcia-Lerma

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Microbicides without ARVs aren't a dead issue, but they're definitely on a resuscitator.  
— Anthony Fauci

many things to learn about optimal [PrEP] dosing,” said Mayer.

Garcia-Lerma also presented data from a new study in rhesus macaques that shows that a single dose of PrEP is not nearly as effective at protecting against a low-dose, repeat SHIV challenge. “The post-exposure dose appears to be essential for protection,” concluded Garcia-Lerma.

The study compared two groups of six monkeys. One group received a single dose of Truvada three days before challenge, while the other received a single dose of the tenofovir pro-drug, GS7340. In Phase I and II clinical trials, GS7340 resulted in 100-fold higher tenofovir concentrations in PBMCs 24 hours after dosing as compared to orally administered tenofovir. All animals, including six controls, were exposed to a low-dose SHIV challenge once a week.

Five of the six macaques that received only a single dose of Truvada became infected after seven challenges, at which time the experiment was stopped. GS7340 didn't fare any better—four of the six animals were infected after four challenges. “This is quite unexpected because of the high drug levels in PBMCs,” said Garcia-Lerma.

When researchers analyzed the GS7340 drug levels, they found no difference between the animals that became infected and those who didn't. They also found that the concentration of the pro-drug in PBMCs persisted for a long time—levels at 17-21 days after dosing were still higher than what is seen following oral administration of tenofovir. High drug levels also were detected in both lymph node and rectal tissues. Additionally, the acute viremia in GS7340-treated animals who became infected was blunted compared to controls, which Garcia-Lerma said was “another indication that the drug was having an effect.”

Yet despite all of this, the drug was unable to protect in this model. Garcia-Lerma said there were many factors that might explain this, including potential differences between the drug's activity at effector and inductor immune sites at the mucosa. He suggested that evaluating the pharmacodynamics of different dosing schedules will be important for determining the most effective PrEP strategy.

### Topical PrEP

In addition to oral dosing, researchers are also studying the use of gel formulation of ARVs that can be used as microbicides. Several efficacy trials of other non-ARV-based microbicides have provided disappointing results, with the latest in a string of failures reported in December 2009

(see *Vaccine Briefs, IAVI Report*, Nov.-Dec. 2009). “Microbicides without ARVs aren't a dead issue, but they're definitely on a resuscitator,” said Fauci. “The time has come to look at an ARV-based microbicide.”

There are two clinical trials underway to evaluate the use of either tenofovir or Truvada as a topical microbicide, with the first results expected later this year. Researchers are also experimenting with other ARVs that may be effective topical PrEP agents. One of these is maraviroc, the first licensed ARV that blocks HIV entry into cells by binding to the CCR5 receptor. Maraviroc was licensed for the treatment of HIV infection by Pfizer, who granted a license for development of the drug as a vaginal microbicide to the International Partnership for Microbicides (IPM).

Neither IPM nor Pfizer planned to evaluate the efficacy of maraviroc in nonhuman primates, according to John Moore, a professor of microbiology and immunology at Weill Cornell Medical College. So, he and his colleagues at the Tulane National Primate Research Center decided to take matters into their own hands. “Success in a monkey model doesn't guarantee it will work in women but if you fail in the monkey, it's not a good sign,” said Moore.

After crushing maraviroc pills and dissolving them in saline to prepare a make-shift microbicide, Moore and colleagues evaluated the efficacy of the vaginally applied compound to protect against a single, high-dose vaginal SHIV162P3 challenge in rhesus macaques, which were previously treated with progesterone to thin their vaginal epithelium.

They found that maraviroc was able to protect the monkeys against this stringent challenge in a dose-dependent manner. Half of the animals were protected at a maraviroc dose of 0.25 mg/ml. The fully protective dose was 4 ml at a concentration of 3 mg/ml. Moore reported that protection also waned over time—the longer the time between application and challenge, the less protective the drug was. The half-life of protection was approximately four hours.

Since a CCR5 inhibitor would obviously not protect against virus that utilizes the CXCR4 receptor to gain entry into cells, Moore and colleagues also conducted a control experiment in which they challenged maraviroc-treated macaques with a high-dose X4-tropic SHIV. As expected, all the animals were infected, but the viral loads in X4-infected animals were the same as in the untreated controls, suggesting to Moore that there was “no indication that maraviroc exacerbates X4 infection.” ■

# Research BRIEFS

## Adding to the Armamentarium of Broadly Neutralizing Antibodies

BROADLY NEUTRALIZING ANTIBODIES (bNAbs) to the HIV Env spike are considered valuable tools for guiding the design of immunogens for AIDS vaccine candidates developed to induce such antibodies. Only a handful of bNAbs—including b12, 2G12, 4E10 and 2F5—were available, until last September, when IAVI researchers, in collaboration with researchers from The Scripps Research Institute in La Jolla, California, reported the isolation of two new bNAbs called PG9 and PG16 (*Science* 326, 285, 2009). Then, at the AIDS Vaccine 2009 conference in October, researchers from the Vaccine Research Center (VRC) at the US National Institute of Allergy and Infectious Diseases (NIAID) reported the isolation of three new bNAbs, including one called VRC01 (see *Raft of Results Energizes Researchers, IAVI Report*, Sep.-Oct. 2009).

Now, a group led by Antonio Lanzavecchia at the Institute for Research in Biomedicine (IRB) in Switzerland, has identified three additional bNAbs from HIV-infected individuals (*PLoS One* 5, e8805, 2010). “Between the three groups, we will have doubled the number of useful antibodies,” says Robin Weiss, a professor emeritus of viral oncology at University College London and one of the researchers involved in this study.

None of the three bNAbs identified in the *PLoS* study “has the potency and breadth combined in a single antibody that say PG9 and PG16 show or that VRC01 shows,” Weiss says. Each of the three antibodies, called HJ16, HGN194, and HK20, recognizes a different part of the Env spike—the CD4 binding site, the tip of the V3 loop, and an epitope on the HR-1 region of gp41, respectively. This suggests “that there are a number of ways in which to neutralize HIV,” says Peter Kwong, chief of the structural biology section at the VRC, who was not involved in the *PLoS* study.

To find the antibodies, the researchers tested sera from about 400 individuals infected with HIV clades prevalent in Africa, such as A and C, for their ability to neutralize a panel of viruses from different clades. They then used Epstein-Barr virus to immortalize the memory B cells of 21 donors with a broad neutralization profile to isolate monoclonal antibodies (mAbs). When they measured binding of these antibodies to recombinant Envelope proteins, including trimeric gp140 (which contains all protein parts of the Envelope spike), monomeric gp120, and gp41, they found that 58 mAbs bound to at least one recombinant Env protein from different clades, including clades A and C.

They then found that more than half of the 58 mAbs neutralized at least one HIV isolate. However, only three were broadly neutralizing, in that they neutralized virus from at least two clades and more than one virus per clade. This suggests that the ability of patient sera to broadly neutralize HIV may often come

from the combined effects of antibodies that are not broadly neutralizing in and of themselves, and only rarely from a few very potent antibodies. “The main message is that there is an extensive number of neutralizing antibodies that can be retrieved from the human memory B cells of infected people, but the breadth of neutralization of these antibodies is very limited,” adds Davide Corti, the study’s first author, who is now director of the antibody discovery unit at Humabs, a spin-off company of the IRB.

Of the three bNAbs, HJ16 is “perhaps the most interesting,” Weiss says. Like b12, it binds the CD4 binding site of gp120, although to a different part. While its breadth is similar to b12, it best neutralizes those strains that b12 does not neutralize. “If you put the two together you get fantastic neutralization,” says Weiss. “[This] is against the dogma that in general monoclonal antibodies neutralize first of all the [neutralization] sensitive viruses and after that some resistant viruses,” Corti says. “It is indeed unusual.”

With the identification of HJ16, there are now three broadly neutralizing antibodies—b12, VRC01, and HJ16—that recognize a different part of the CD4 binding site, says Dennis Burton, a professor of immunology and microbial science at The Scripps Research Institute, who was involved in the PG9/16 study. “It adds to our knowledge about the CD4 binding site and it will help us to design [vaccine] candidates based around the CD4-binding site,” Burton says.

The second antibody, HGN194, recognizes a very conserved epitope, Corti says, in the V3 loop of gp120, which interacts with the CCR5 coreceptor during the HIV entry process. The V3 epitope consists of a continuous stretch of amino acids, which may make immunogen design for this type of antibody specificity easier, he adds.

The third antibody, HK20, binds to a region of gp41 that was thought to be inaccessible because it is close to the viral membrane and only exposed transiently, just before fusion takes place. This is probably why HK20 only becomes a potent and broadly neutralizing antibody as an Fab fragment, says Corti.

The method used to isolate PG9/16 involved screening for neutralization first. But Corti and colleagues identified their crop of bNAbs by first screening for their ability to bind recombinant Env protein and only later for their ability to neutralize HIV. “We now think that that’s not the smartest way to do it,” Weiss says, because an approach that first screens for binding might miss antibodies that can neutralize HIV even though they don’t bind well to recombinant Env proteins. His group is now screening for neutralization first, Weiss says. “There are better robotic methods to pick up neutralizers, and so over the last year it’s become much easier to do screening by primary neutralization.” —*Andreas von Bubnoff*

# Vaccine BRIEFS

## Ushering in the Decade of Vaccines

THE BILL & MELINDA GATES FOUNDATION, which has made disease prevention a cornerstone of its philanthropic efforts, announced a US\$10 billion commitment over 10 years to fund research, development, and distribution of vaccines to people in the world's poorest countries. The *Chronicle of Philanthropy* said it was the largest pledge ever by a grant-maker for a specific cause.

The \$10 billion pledge is in addition to the \$4.5 billion already committed by the Gates Foundation for research and development of new vaccines—including AIDS vaccine research—and delivery of existing vaccines. The Foundation said its increase in vaccine funding was inspired by the remarkable progress in recent years in improving access to existing vaccines and the introduction of new vaccines against rotavirus and pneumococcal disease. The World Health Organization estimates that pneumonia and rotavirus infection, a cause of severe diarrhea, together account for 1.3 million deaths every year in children under age five, mostly in developing countries.

"We must make this the decade of vaccines," said Bill Gates, after he announced the substantial donation during the World Economic Forum's annual meeting in Davos, Switzerland, which took place from January 26-31. "Vaccines already save and improve millions of lives in developing countries. Innovation will make it possible to save even more lives."

Julian Lob-Levyt, executive secretary of the GAVI Alliance, a Geneva-based non-profit organization that partners with drug companies, health agencies, and charities to provide both financial and programmatic support for vaccination pro-

grams in 73 of the poorest countries in the world, noted that the Foundation's \$10 billion pledge set a new precedent in global health.

"This is a fantastic announcement," says Lob-Levyt. "It gives us great momentum." But Lob-Levyt also warned that this funding won't be enough. He said he hoped the Gates pledge would spur other public and private donors to expand their support. "Vaccines remain the most cost-effective way of saving children's lives," says Lob-Levyt.

The decision to pour more money into vaccines is based on mathematical models that suggest scaling up the delivery of life-saving vaccines in developing countries to 90% coverage—including the new rotavirus and pneumococcal vaccines—would prevent the deaths of an estimated 7.6 million children under age five by 2019. The Gates Foundation also estimates that an additional 1.1 million children could be saved with the rapid introduction of a malaria vaccine beginning in 2014. A Phase III efficacy trial of GlaxoSmithKline (GSK) Biologicals' RTS,S malaria vaccine candidate began last year. If the results of this trial are encouraging, the candidate vaccine could be submitted to the European Medicines Agency for regulatory review by 2011 and be ready for distribution by 2012, according to GSK and the Malaria Vaccine Initiative.

Lob-Levyt stressed that a portion of the Gates Foundation's latest commitment will be used to support the search for new vaccines, including AIDS vaccine research, although he said it is not known at this point how much of the money will go to research into new vaccines and how much will be spent on expanding access to existing vaccines. —Regina McEnerly

## Journal Retracts Controversial Article that Spurred Anti-vaccine Sentiment

A CONTROVERSIAL 1998 RESEARCH PAPER in *The Lancet* that attempted to link the onset of a number of behavioral symptoms with the mumps, measles, and rubella (MMR) vaccine in a handful of children—prompting an abrupt decline in childhood immunizations—was retracted after a UK panel determined that the authors who conducted the study acted unethically (*Lancet* 351, 637, 1998).

A one-paragraph statement released by the editors of *The Lancet* on February 2 said the judgment by the UK General Medical Council's Fitness to Practice Panel in January prompted their deci-

sion to retract the paper (*Lancet* 375, 445, 2010). "It was utterly clear, without any ambiguity at all, that the statements in the paper were utterly false," Richard Horton, editor-in-chief of *The Lancet* told the British newspaper *The Guardian* in February, the same day his journal issued the retraction. "I feel I was deceived."

The 1998 research paper described an unexpected pattern of chronic enterocolitis in 10 of 12 children with developmental disorders. The authors of the study said the intestinal lesions occurred, in most cases, after the children received the MMR

vaccine, which is typically given by 15 months of age. The paper also cited previous, unrelated studies that attempted to link patterns of colitis and another intestinal disorder, ileal-lymphoid-nodular hyperplasia, with sudden behavioral changes, including development or autism spectrum disorders in young children.

The study did not prove any causation between vaccination against MMR and intestinal disorders. Yet, Anthony Wakefield, a researcher from the Royal Free Hospital and School of Medicine in London who led the study, held a press conference following publication of the study, at which he urged parents to shun the combination MMR vaccine in favor of having their children vaccinated with the three vaccines individually, with a year interval between each dose. Ten of the paper's 13 authors—not including Wakefield—submitted a partial retraction in 2004 saying they felt research into the intestinal lesions should continue, but stressed that the paper established no causal link between the MMR vaccine and autism (*Lancet* 363, 750, 2004). “The possibility of such a link was raised and consequent events have had major implications

for public health,” the authors wrote in the retraction.

The controversial paper is widely credited with sparking an anti-vaccination movement that resulted in declines in immunizations, particularly in the UK. In 1997, the year before the study was published, 91% of children in the UK were vaccinated. In 2003, the rate had dropped to 60% in some parts of the country.

Following the retraction by *The Lancet* in February, the US Centers for Disease Control and Prevention (CDC) released a statement reminding parents that vaccines are safe, effective, and that they save lives. “*The Lancet’s* retraction of Dr. Wakefield’s study is significant,” the CDC noted. “It builds on the overwhelming body of research by the world’s leading scientists that concludes there is no link between the MMR vaccine and autism.”

Jerome Kassirer, editor-in-chief of the *New England Journal of Medicine* from 1991-1999, said this was an unusual and unfortunate event. “It terrifies me that people are scared of vaccinating their children. There is a lot of fear and I think it is all a manifestation, in large part, of irrational reasoning,” said Kassirer. —*Regina McEnery*

## 2011 US Budget Proposal Calls for Increase in HIV/AIDS Spending

DESPITE A THREE-YEAR SPENDING FREEZE being sought for many of the country’s domestic programs, US President Barack Obama unveiled a US\$3.6 trillion budget proposal for fiscal year 2011 that includes a \$1.2 billion increase in HIV/AIDS spending. The US Congress will consider the proposed budget request during a series of hearings later this year, and is expected to finalize the 2011 budget before it kicks in on October 1, 2010.

The \$27.2 billion being sought for HIV/AIDS programs includes \$2.7 billion for AIDS research allotted to the US National Institutes of Health (NIH)—a 3.1% increase from the 2010 budget—most of which will be allocated to the National Institute of Allergy and Infectious Diseases (NIAID). Obama’s budget proposal also allocates \$4.8 billion in funding for NIAID, a 3.3% increase from this year. How much of that will be directed to AIDS vaccine research is unclear; however, NIAID has indicated in its 2011 budget justification document that there will be a “renewed focus” on vaccine discovery research and “continued support for the research of HIV pathogenesis, including the search for novel approaches that interrupt HIV transmission and studies that take a systems biology approach to understand the complex interactions between HIV and the immune system.”

Obama’s proposed budget also reflects an increased allotment for domestic HIV/AIDS programs, including a \$31 million increase for HIV prevention programs at the US Centers for Disease Control and Prevention, a 4% increase over this year, as well as \$9.6 billion for global health funding—a 9% increase from 2010. This includes a 2.6% increase in funding for the President’s Emergency Plan for AIDS Relief (PEPFAR), the mammoth HIV prevention, treatment, and care program that

has brought ARV therapy to 2.4 million people in 30 countries. PEPFAR, which was begun under the previous administration, is the main plank in a \$63 billion Global Health Initiative (GHI) that was announced shortly after Obama took office in 2009.

The GHI aim is to put more than four million people on ARVs and prevent more than 12 million new HIV infections by 2014. But the premise of the GHI is also to take a more integrated approach to global health, and to tackle other global problems such as reducing maternal mortality, improving childhood nutrition, and combating neglected tropical diseases.

At the recent 17th Conference on Retroviruses and Opportunistic Infections in San Francisco, US Global AIDS Coordinator Eric Goosby said PEPFAR is the cornerstone of GHI, but he said the time has come to shift PEPFAR out of an “emergency response and into a more sustained response.”

“We hope to begin to talk about a shared responsibility and a global responsibility in the move to universal access,” said Goosby, during his address.

But groups advocating for increased spending for HIV/AIDS say the amount of money the Obama administration intends to spend next year, particularly to combat the AIDS pandemic in developing countries, is not enough. “PEPFAR has been a forceful engine driving down AIDS mortality, heading off new infections and extending lifesaving drugs to millions of HIV patients,” said Kenneth Mayer, co-chair of the Center for Global Health Policy’s Scientific Advisory Committee. “Unfortunately, this budget could imperil the fragile gains made over the last decade in treating HIV. It could also force a Sophie’s Choice between prevention and treatment.” —*Regina McEnery*

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