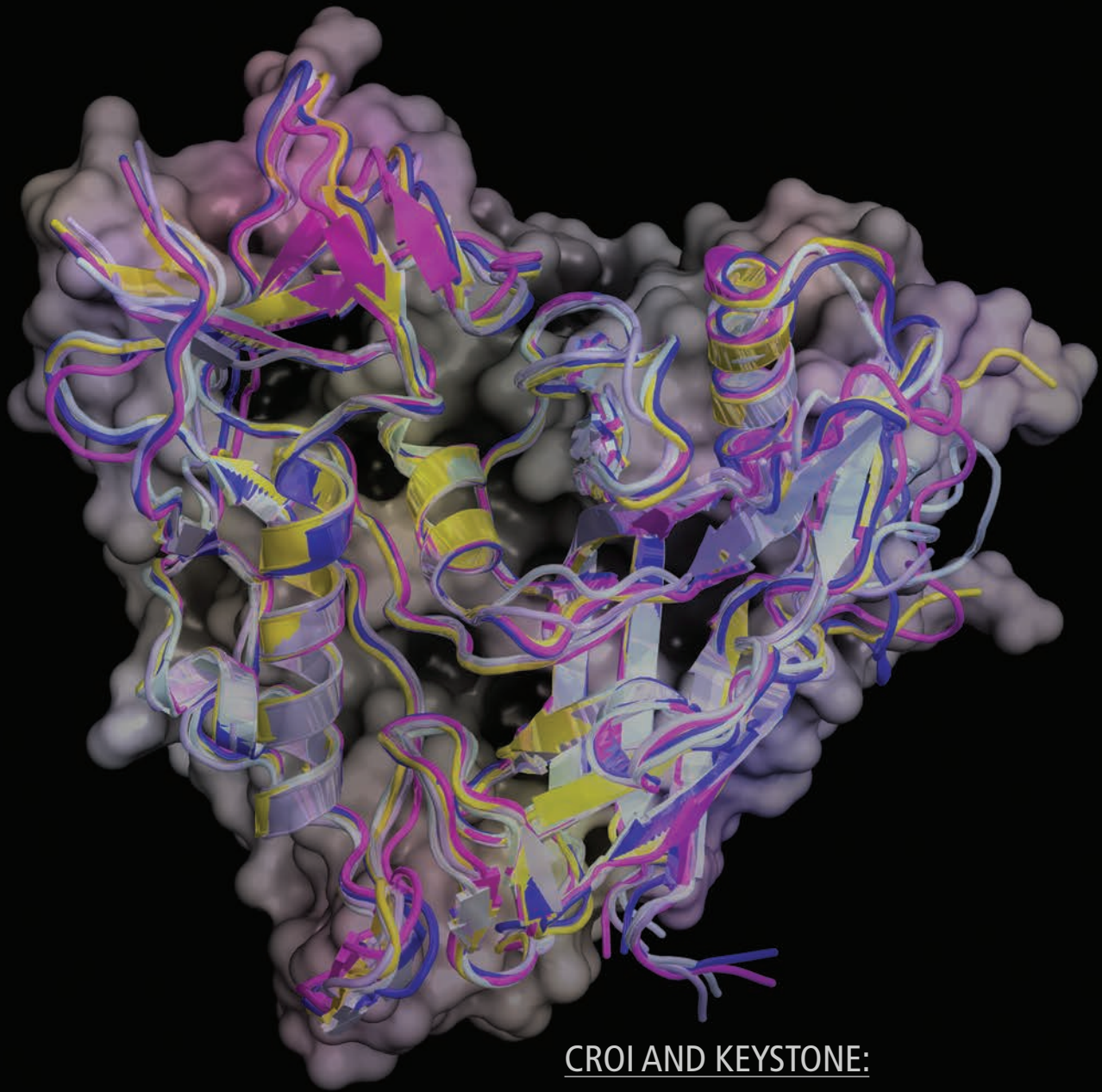


MARCH-APRIL 2012

IAVIRreport

The Publication on AIDS Vaccine Research

WWW.IAVIREPORT.ORG | VOLUME 16, NUMBER 2



CROI AND KEYSTONE:

**CONFERENCE
CAROUSEL**

EDITOR'S LETTER

When managing editor Kristen Jill Kresge informed her staff that she was leaving the editing of *IAVI Report* in my hands while she went off to have a baby, I imagine their response was, well, polite. Or maybe not: Kristen is, as subscribers to this magazine doubtless know, a tough act to follow, even temporarily. Fortunately for me, she had the grace—and good sense—to plan out the issue before she went on leave.

Aware that major HIV conferences were looming, Kristen dispatched her reporters to cover them. You will notice that they did so with gusto. Our sweeping report from the 19th Conference on Retroviruses and Opportunistic Infections (CROI) may not qualify as light summer reading, but it does have a little something for everybody (see page 4). It recounts how researchers are harnessing new technologies to solve the structure of the recalcitrant HIV spike, and shares insights gleaned from the continuing analysis of samples collected during the RV144 trial, which demonstrated for the first time that a vaccine can prevent HIV transmission. Another segment delves into similar analyses of trials evaluating antiretroviral treatment as a mode of prevention. For those with an appetite for basic biology, the article includes absorbing descriptions of HIV's interaction with an elusive subclass of T helper cells, and research parsing the evolution of HIV's intracellular defenses.

Our articles on the Keystone Symposia this season are much more focused. We felt that broadly neutralizing antibodies stole the limelight at the Keystone HIV Vaccines meeting, and that's reflected in our report (see page 10). The article takes a tour through the structural, biochemical, and genetic analyses of these potent molecules, and details how the latest findings are inspiring new HIV vaccine strategies. The story out of the Keystone meeting on HIV Pathogenesis, meanwhile, pivots to the search for a cure for HIV (see page 15). It relates progress in efforts to locate and eradicate latent HIV sequestered in cells, and covers clinical studies that seek to safely replicate the conditions that cured Timothy Brown—the only person known to have cleared an HIV infection.

If all this isn't enough science for you, the issue has its usual complement of Research Briefs (see page 22). If it is, we also have stories on new treatment guidelines from the WHO, and the appointment of a new director for the Global HIV Vaccine Enterprise (see page 20).

Kristen may have planned out this issue, but I've certainly enjoyed editing it. I hope you will take as much pleasure in its perusal.

– UNMESH KHER



All rights reserved ©2012

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, IAVI works with partners in 25 countries to research, design and develop AIDS vaccine candidates. In addition, IAVI conducts policy analyses and serves as an advocate for the AIDS vaccine field. IAVI supports a comprehensive approach to addressing HIV and AIDS that balances the expansion and strengthening of existing HIV-prevention and treatment programs with targeted investments in the design and development of new tools to prevent HIV. IAVI is dedicated to ensuring that a future AIDS vaccine will be available and accessible to all who need it. IAVI relies on the generous donations from governments, private individuals, corporations and foundations to carry out its mission. For more information, see www.iavi.org.

IN THIS ISSUE

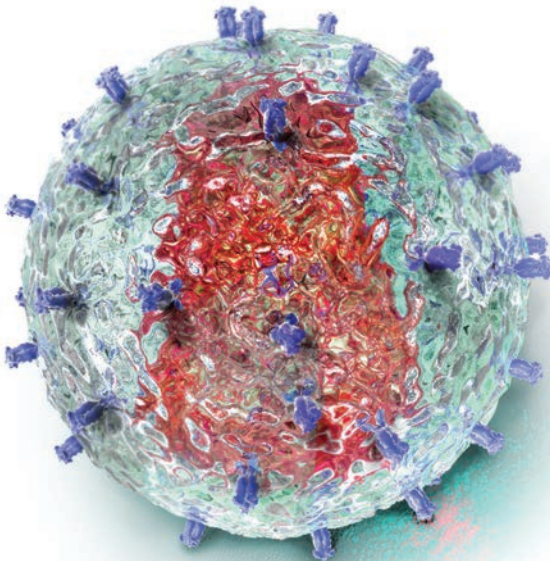
04 **A Slew of Science in Seattle**
CROI served up a snapshot of HIV's Envelope trimer and the latest on PrEP and protective immune responses.

10 **Tapping the Sanguine Humor**
Broadly neutralizing antibodies stole the show at Keystone.

15 **Stalking HIV's Sleeper Cells**
This year's HIV Pathogenesis meeting focused on latent HIV reservoirs—and how they might be eradicated.

20 **Vaccine Briefs**
WHO Updates Guidelines on ARV Treatment; Global HIV Vaccine Enterprise Appoints New Director.

22 **Research Briefs**
Predicting the Antiviral Activity of Three-drug Combinations in HAART; CD4⁺ T Cells Can Control Viral Load by Directly Killing HIV-infected Cells.



IAVIReport

MANAGING EDITOR

Kristen Jill Kresge

SENIOR SCIENCE WRITER

Andreas von Bubnoff, PhD

SCIENCE WRITER

Regina McEnery

PRODUCTION & WEBSITE MANAGER

Nicole Sender

FREE SUBSCRIPTIONS:

To obtain a FREE print subscription to *IAVI Report*, change your subscription details, or receive multiple print copies of *IAVI Report* to distribute and/or use in your programs, please go to www.iavireport.org and click on the Subscribe link.

For more information, go to:

www.iavireport.org

Comments/Questions?

Email us at iavireport@iavi.org

[ON THE COVER]

Ribbon diagram (outlining the protein backbone) of crystal structures of HIV-1 gp120 core, the part of HIV's Envelope spike that lacks the transmembrane gp41 part and the variable loops, and that binds to the CD4 receptor on the target cell. The yellow structure is the conformation gp120 assumes when bound to the CD4 receptor. The pink, blue, lilac and white structures are recently determined conformations of four gp120 cores not bound to CD4, taken from four different HIV-1 strains (*Proc. Natl. Acad. Sci.* **109**, 5663, 2012). The close similarity of the unbound to the CD4-bound structure surprised researchers, because it contrasts with the very different shape that unbound gp120 assumes when the other parts of the Env spike such as gp41 and the variable loops are present. This indicates that in the natural, unbound Env spike, these other parts of the Env spike keep gp120 in a shape that's different from the shape it needs to assume to bind CD4, probably to enable HIV to evade recognition by the immune system.

Image courtesy of Jonathan Stuckey (Vaccine Research Center, NIAID, NIH).

A Slew of Science IN SEATTLE

CROI served up a snapshot of HIV's Envelope trimer and the latest on PrEP and protective immune responses

By Regina McEnery and Richard Jefferys

As the global campaign against AIDS enters its fourth decade, the development of a broadly preventive HIV vaccine remains among its most vexing challenges.

In his opening remarks at the 19th Conference on Retroviruses and Opportunistic Infections (CROI), held March 5-8 in Seattle, Tufts University virologist John Coffin, the scientific organizer of the event, noted that the failure of previous vaccine candidates had convinced many scientists that antibodies capable of preventing HIV infection could not be elicited through vaccination.

But the 31.2% efficacy demonstrated in the RV144 vaccine trial in Thailand—which, though modest, provided the first evidence of vaccine-induced protection from HIV—has helped lift the gloom from such speculations. Volunteers in that trial who received the prime-boost vaccine combination developed low titers of gp120-binding antibodies that subsequent analyses revealed are correlated with the risk of HIV infection. Separately, a dramatic expansion in the number of broadly neutralizing antibodies (bNAbs) against HIV isolated from volunteers, and the data describing their mechanisms of action, have renewed optimism about the prospects of this vaccine strategy.

“We’re now thinking much more seriously about developing vaccines that might be based on eliciting specific antibodies,” Coffin told the international gathering of more than 4,200 HIV researchers and clinicians.

Reflecting this shift in strategy, CROI organizers selected pioneering antibody researcher Dennis Burton, professor of immunology and microbial science and director of IAVI’s Neutralizing Antibody Center (NAC) at The Scripps Research Institute in La Jolla, California, to kick off the confer-

ence. Burton observed that the recent discovery of more than two dozen potent bNAbs by his lab and others, and the elucidation of some of their structural targets on HIV’s Envelope glycoprotein, have revealed weaknesses that can be exploited for both drug and vaccine development. “The tools are all there,” said Burton. “It remains to be seen if immunogen design can take advantage of all these tools.”

But antibodies were far from the only item on CROI’s four-day agenda. The conference also highlighted investigations of the structure of HIV’s Envelope trimer and updates on the continuing analysis of samples collected in the RV144 trial. Other talks of particular interest covered new findings on how a subset of T cells influences antibody development, the evolutionary pathways of HIV and SIV, and the results of several recent studies on ARV-based prevention, which dominated the conference and provoked more than a few animated discussions.

Freezing the fidgety trimer

All known bNAbs against HIV target functional Envelope trimers that—rather sparsely—adorn the viral surface. Many researchers believe that obtaining the detailed molecular structure of this protein complex would bring us much closer to the design of vaccines that induce sterilizing immunity to HIV, the ultimate goal of the AIDS vaccine field.

But that objective is easier defined than attained. The spike—composed of three identical pairs of the extracellular gp120 and transmembrane gp41 proteins—is highly unstable and structurally dynamic. While a structure of the HIV Envelope glycoprotein gp120 bound to CD4 was obtained more than a decade ago through X-ray crystallography (*Nature* 393, 648, 1998), scientists haven’t yet obtained a high-resolution structure of the unliganded trimer.

In a riveting plenary talk describing his use of cryo-electron microscopy (cryo-EM) to probe the trimer's structure, Joseph Sodroski, professor of microbiology and immunology at the Dana-Farber Cancer Institute, Harvard Medical School, compared the Envelope protein in its unliganded state to a mousetrap that has been set but not sprung. Once it binds its receptors on the surface of the T cell, the trap is sprung. At that point its gp120 and gp41 components each assumes a "preferred, lower energy" conformation. "The difference in energy between starting and final conformation," Sodroski explained, "is used to drive the fusion of viral and target cell membranes, and complete the cell entry process."

But the only low-resolution images available are of the unliganded HIV Envelope trimer and a brief subsequent state, assumed just before viral entry, known as the prehairpin intermediate. Sodroski said the main problem is that researchers haven't yet been able to generate crystals of the functional Envelope protein that are amenable to X-ray crystallography. The resolutions achieved using electron tomography, meanwhile, are too low to reveal the details essential to protein engineering.

So Sodroski's lab turned about three years ago to single-particle analysis of the HIV trimer using cryo-EM. This approach, said Sodroski, permits near atomic-level resolution of molecular structures, allowing researchers to generate reconstructions of molecular assemblies that resist crystallization.

Sodroski and colleagues first expressed the Envelope glycoproteins obtained from a primary HIV-1 strain in JR-FL cells, and then fragmented their membranes (which carried the intact trimer) with detergent to release the soluble proteins. They then purified and froze the molecules, spread them out in thin layers and obtained multiple micrographs of the particles from a variety of angles. To improve the resolution and reduce background noise, they averaged images of particles in similar orientations taken from multiple angles. They then aggregated the results to generate a structure of the trimer.

Sodroski said close to a million single-particle images were used to create the 3D image of the Envelope trimer at nearly atomic-level resolution. The resulting single-particle cryo-EM structure agreed with a structure of the native Envelope protein trimer constructed by cryo-electron tomography in the laboratory of Sriram Subramaniam, chief of the Biophysics Section in the Division of Cell Biology at the US National Cancer Institute (see *The Beauty Behind the Beasts*, *IAVI Report*, Nov.-Dec. 2009).

A striking architectural feature of the HIV

Envelope trimer in its unliganded state, Sodroski said, is the "doughnut hole" at its center. This conformation, he noted, is distinctly different from the densely packed structure that emerges at the end stage of the viral entry process. The images obtained from cryo-EM show that the gp41 protein is kept from its more energetically favorable conformation by the inner domain of gp120, which acts like a clamp holding the membrane-distal domains of the former far apart. Sodroski pointed out that the unliganded gp120 protein also has an open structure. The only parts of that molecule that interact with each other are the trimer association domains—the V1, V2 and V3 loops.

Sodroski said that the architecture of the unliganded HIV Envelope glycoprotein suggests that broadly neutralizing antibodies must not only recognize conserved epitopes, but approach them from appropriate angles as well. He noted that more detailed reconstructions of the trimer structure could aid the design of immunogens that can elicit similar antibodies.

Of sieves and the sifting of samples

The continuing correlates analysis of samples collected in the RV144 trial provided some grist for discussion as well. Paul Edlefsen, a biostatistician at the Public Health Sciences and Vaccine and Infectious Disease Division of the Fred Hutchinson Cancer Research Center in Seattle, added a little texture to the primary analysis of RV144 samples. That analysis suggested that the presence of non-neutralizing IgG antibodies that bind to the V1/V2 loops of HIV Env correlated with a 43% reduction in HIV infection risk in vaccine recipients (see *A Bangkok Surprise*, *IAVI Report*, Sep.-Oct. 2011).

Last year, his team, in collaboration with teams from the US Military HIV Research Program (MHRP) and the University of Washington, presented an analysis of breakthrough viruses—those that emerge in individuals who are not protected by vaccination— from 110 infected recipients across the vaccine and placebo arms of the RV144 trial. Their analysis compared nearly 1,000 amino acid sequences from the envelope proteins of Clade E HIV-1, the dominant subtype contained in the AIDSVAX B/E gp120 boost, from 50 vaccinees and 71 placebo recipients in the trial. These studies, led by MHRP virologists Morgane Rolland and Sodsai Tovanabutra and University of Washington virologist Jim Mullins, found viral escape (known to vaccinologists as a "sieve effect") to be associated with the V2 and C1 region of the Envelope protein.

This is of particular interest because C1 cor-

[A BOOST IN THEIR PRIME]

After decades of stagnation through much of the 20th century, research into adjuvants—substances that boost immune responses to vaccines—is enjoying a boom of sorts, fueled in part by improved understanding of the interplay between the innate and adaptive immune responses. At CROI, a team from the Atlanta biotech GeoVax offered up a plum example of this phenomenon.

GeoVax has devised an immunization regimen in which the gene for GM-CSF, a cytokine produced by macrophages, neutrophils, and a variety of other immune cells, is co-expressed as an adjuvant with a SIVmac239 DNA prime. They have previously shown that, following such priming, a boost with SIVmac239 MVA prevents acquisition of simian immunodeficiency virus (SIV) by 70% of macaques subsequently challenged a dozen times with SIVsmE660.

In their latest study, the team examined the durability of that effect. Seven of the macaques who did not acquire SIV following rectal challenge in the initial study—two who did not receive the GM-CSF adjuvant and five who did—were boosted with only the MVA vaccine candidate. They were then re-challenged six months later with 12 weekly doses of SIVsmE660.

GeoVax's chief scientific officer Harriet Robinson reported that six macaques remained uninfected after the challenge, while the seventh animal—from the adjuvanted group—acquired SIV only after the 10th challenge. The late MVA boost, she said, increased CD4⁺ and CD8⁺ T-cell responses to levels similar to early peak responses, and boosted antibody responses to the SIV envelope protein to four times the levels observed in earlier peak responses.

Robinson and her team suggest that if their DNA/MVA prime-boost regimen includes a GM-CSF adjuvant, a yearly MVA boost could provide long-lasting protection against repeated rectal challenge. GeoVax is now testing the safety and immunogenicity of a GM-CSF adjuvanted DNA/MVA HIV vaccine candidate in a Phase I trial (HVTN 094) that opened for enrollment in April. —RM

responds to a common antibody-dependent cellular cytotoxicity (ADCC) epitope, and ADCC is thought to have played a role in the protection induced by the RV144 regimen. The sieve effect appeared to be associated with a region between amino acids 69-95 in the C1 region. They identified two amino acid positions that appear to help determine whether a given clade E HIV variant escaped the nominal protection afforded by the RV144 vaccine regimen. The amino acids at positions 169 and 181 that correlated with such escape correspond to the crown of the V2 loop and the third amino acid of the $\alpha 4\beta 7$ binding motif, respectively (see *A Bangkok Surprise, IAVI Report*, Sep.-Oct. 2011).

Edlefsen shared this year the results of a linear peptide microarray assay—developed by Duke University scientist David Montefiori—that his laboratory used to conduct its secondary correlates analysis of RV144 samples. The assay was designed to detect binding of antibody in the vaccinees' sera to linear epitopes representing all clades of HIV-1. This showed that people who have antibodies binding to the crown of the V2 loop were less likely to become infected, confirming results of the primary correlates analysis. The detection of IgG binding to linear peptides, he argued, strengthens the relevance of V2 antibody

responses observed in the initial correlates studies.

Other findings have similarly boosted the starring role of the V2 loop in RV144. Edlefsen discussed, for example, two novel human monoclonal antibodies (mAbs), CH58 and CH59, retrieved from blood samples collected from a vaccine recipient after the fourth vaccination in the RV144 regimen. The mAbs—cloned by the lab of Duke University scientist Barton Haynes, who led the scientific steering committee that oversaw the search for RV144 correlates—are only weak neutralizers of HIV, unlike the PG9 bNAb, which they resemble. They do, however, bind the crown of the V2 loop implicated by sieve analysis. Interestingly, a substitution of the amino acid alanine at position 169 of the V2 loop abrogates this binding, but a similar substitution at position 181 does not. Exactly why that may be remains unclear.

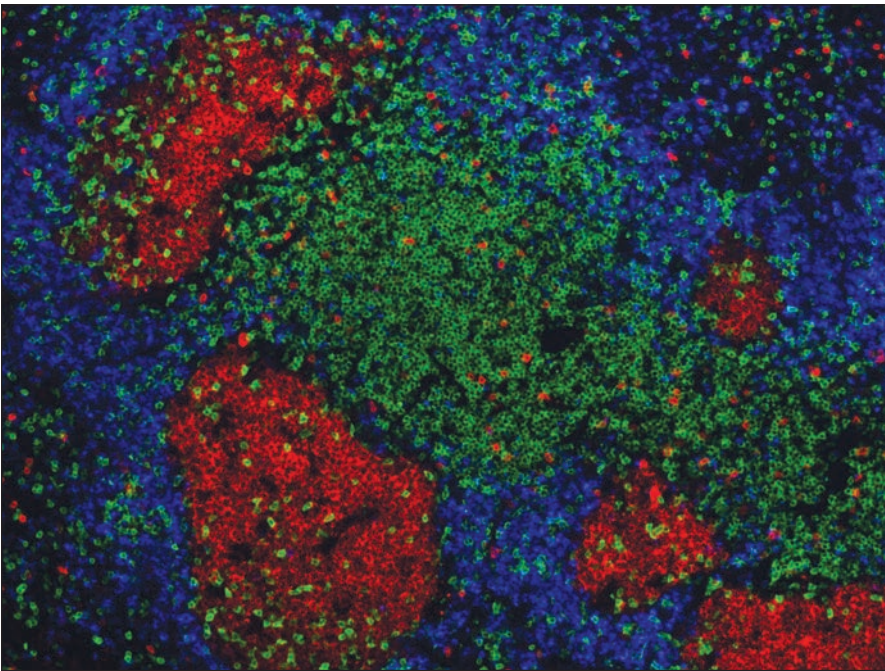
HIV and the elusive follicular helper

Immunologists have long been aware that CD4⁺ T cells—which are T-helper cells—play a vital role in the antibody response. One of those roles is the delivery of signals that drive the maturation and selection of B cells that generate increasingly potent antibodies. This process, called affinity maturation, is critical to the evolution of bNAbs to HIV.

But only in the last decade has it become clear that there exists a specialized subset of CD4⁺ T cells that is dedicated to such maturation. Carola Vinuesa, Humoral Immunity & Autoimmunity Group Leader at the John Curtin School of Medical Research, Australian National University in Canberra, spoke to the CROI audience about this subset—T follicular helper (Tfh) cells. Part of the reason Tfh cells eluded identification for so long is that they hang around in the B-cell follicles of lymph nodes and tend to be poorly represented in the circulating population of immune cells.

Vinuesa explained that the discovery of Tfh was facilitated by the identification of the chemokine receptor CXCR5 in the mid-1990s (*Cell* 87, 1037, 1996). This receptor was shown to be required for lymphocyte migration into lymph node follicles and the formation of germinal centers, where B-cell proliferation and affinity maturation of antibodies occur. Subsequent work by several research groups revealed that high levels of CXCR5 expression defined a subset of CD4⁺ T cells that home in on lymph node follicles and provide help to B cells (*J. Exp. Med.* 192, 1545, 2000, *J. Exp. Med.* 192, 1553, 2000).

The elevation of these Tfh cells to the rank of an independent subset was initially boosted by data



T follicular helper cells in action. In the red germinal center (stained for cytidine deaminase, a marker for germinal center B cells), T follicular helper cells (stained green for CD3) can be seen interacting with B cells. An outer ring of re-circulating follicular B cells (stained blue for IgD), known as follicular B-cell mantles, surround the germinal center. Other T-cell zones are stained green as well. Image courtesy of Shimpei Kawamoto and Carola Vinuesa.

revealing that their gene expression patterns are distinct from that of better-known Th1, Th2 and T-regulatory groups of the CD4⁺ T-cell clan (*J. Immunol.* 173, 68, 2004). It was subsequently cemented by the identification of the Bcl-6 gene as the master regulator of their differentiation (*Immunity* 31, 457, 2009). If any doubt lingered about the legitimacy of the promotion, it was dispelled by the finding that, in addition to CXCR5, Tfh cells are distinguished by the expression of high levels of the PD-1 molecule and that they primarily secrete the cytokine IL-21.

Vinuesa highlighted two types of interactions that occur between Tfh and B cells: a brief dalliance at the border of the lymph node follicle and germinal center that leads to the generation of short-lived antibody-producing plasma cells and promotes class-switching of antibodies—from the IgM class to mature IgG, IgA or IgE classes—and longer, secondary liaisons in the germinal center that facilitate affinity maturation and the development of long-lived memory B cells. The secondary interface is crucial to the induction of lasting memory responses

A Tip of the Hat to Cure Research

There was a time when the notion that HIV infection might be cured was considered quixotic at best in scientific circles. That is no longer the case. One measure of how much things have changed was the first ever dedicated inclusion in this year's CROI of a symposium covering advances in that quest. The crowded event featured overviews of the state of the science, and much of the same material covered in the recent HIV Persistence Workshop in St. Maarten (see *In Pursuit of a Cure, IAVI Report*, Jan.-Feb. 2012), including a study by David Margolis, director of the University of North Carolina School of Medicine, that evaluated the activity of a single dose of SAHA (vorinostat) in humans.

Sharon Lewin, Professor and Director of Infectious Diseases, Alfred Hospital and Monash University in Melbourne, offered a glimpse at a similar trial she is currently leading. But unlike Margolis' study, hers involves 14 days of vorinostat administration. Ten of the desired 20 participants have so far enrolled in her trial. They have a median CD4⁺ T-cell count of 710, and average around seven years on suppressive ART. Due to the repeat dosing, grade 1 and 2 adverse events have been common, including lethargy, nausea, vomiting, diarrhea, thrombocytopenia (decreased platelet counts) and increased levels of the enzyme alkaline phosphatase. Median onset of side effects was 2-3 days into the dosing period. Those side effects, however, did not persist once the participants completed the necessary 14 days of treatment. No evidence of T-cell activation has been observed. Data measuring the effects of the regimen on latent HIV reservoirs are pending, and will likely be available next year.

One of the unanswered questions in cure research has been whether induction of HIV RNA expression in latently infected CD4⁺ T cells will suffice to induce cell death. At CROI, Liang Shan, a graduate student in the laboratory of Robert Siliciano at Johns Hopkins, offered a sobering answer: latently infected CD4⁺ T cells do not die after exposure to vorinostat, but require functional HIV-specific CD8⁺ T cells to deliver the *coup de grace*. Shan studied whether HIV-specific CD8⁺ T cells sampled from several different groups—naïve controls, HIV-infected individuals on suppressive ART and elite controllers—could kill latently infected CD4⁺ T cells treated with vorinostat in the laboratory.

CD8⁺ T cells from three elite controllers went about this task with vigor, but only one out of eight individuals on ART showed similar activity, indicating that HIV-specific CD8⁺ T-cell dysfunction in chronic infection will need to be addressed if elimination of latently infected

CD4⁺ T cells is to be achieved. Shan reported that stimulation of the CD8⁺ T cells with HIV antigens prior to mixing with the infected CD4⁺ T cells restored their lethality, suggesting that therapeutic HIV vaccines may be an important component of anti-latency strategies. Shan's work was published shortly after his presentation (*Immunity* 36, 491, 2012).

While one goal of cure research is to eradicate HIV, there is also interest in the possibility of a "functional cure," defined as control of the virus and prevention of disease progression in the absence of ART. In support of that effort, Emmanouil Pappasavvas, senior scientist in Luis Montaner's laboratory at the Wistar Institute in Philadelphia, evaluated the effects of pegylated alpha interferon—a cytokine therapy used to treat hepatitis C—on HIV viral load following interruption of ART. A total of 20 participants on suppressive ART with CD4⁺ T-cell counts over 450 were recruited and, after eight weeks, randomized to receive one of two pegylated alpha interferon doses given weekly. After an additional five weeks, ART was interrupted for up to 24 weeks before being restarted, while pegylated alpha interferon was continued as monotherapy. The primary endpoint of the study was the proportion of participants with viral loads less than 400 copies/ml at week 12 following interruption of ART.

Intriguingly, nine out of 20 participants maintained control of viral load below this level at the week 12 timepoint, a percentage far higher than that obtained in prior ART interruption studies. Pappasavvas showed that this salutary outcome was associated with the ability of the participant's natural killer (NK) cells to respond to alpha interferon signaling. Binding of alpha interferon to its cellular receptor typically leads to a cascade of signals that cause phosphorylation of a protein called STAT1, which in turn translocates to the nucleus and steps up transcription of alpha interferon-stimulated genes, driving antiviral responses. Pappasavvas measured the ability of participant NK cells to phosphorylate STAT1 after exposure to alpha interferon *in vitro* and found that such activity correlated significantly with the control of viral load observed in participants ($p=0.005$). A similar association was observed for measures of NK cell cytotoxicity. The results suggest that alpha interferon signaling pathways can play a key role in the immunological control of HIV. Although pegylated alpha interferon has an infamous array of severe side effects—including depression, nausea, vomiting and neutropenia—likely to dampen enthusiasm about its use as a monotherapy, Pappasavvas's work points to novel mechanisms that may have the potential to be exploited by researchers pursuing a functional cure. —RJ

[SHADES OF HLA]

Not all people respond to HIV infection in the same way. For one thing, some control the amount of virus in their bodies far better than others. Many genetic idiosyncrasies—in both the virus and the host—account for such differences. Srinika Ranasinghe, a post-doc in Hendrik Streeck's lab at The Ragon Institute, reported one such factor: variations in a key set of host genes encoding the human leukocyte antigen (HLA).

The Streeck lab has recently illuminated associations between the breadth of HIV-specific CD4⁺ T-cell responses and the control of viral load in chronic infection (*J. Virol.* **86**, 277, 2012). It also has exposed the impact of cytotoxic activity of HIV-specific CD4⁺ T cells in acute infection on both set-point viral load and clinical outcomes (see *Research Briefs*, this issue, and *Sci. Transl. Med.* **4**, 123ra25, 2012).

At CROI, Ranasinghe unveiled new data describing how various HLA alleles might influence CD4⁺ T-cell responses and, as a consequence, viral load. HIV peptides are presented to CD4⁺ T cells by class II HLA proteins, which are encoded by highly variable genes.

Samples from 1,085 treatment-naïve HIV-infected individuals with a mean viral load of 40,472 copies/ml were used for the study. All were of European ancestry and results were adjusted for the presence of class I HLA alleles known to influence viral load, such as B*57, B*27 and B*35px. Examples of alleles associated with lower viral load included HLA DRB1 1502 and HLA DRB1 1302, while HLA DRB1 1301 was linked to higher viral load. Further analysis revealed that HLA alleles associated with lower viral load were more versatile, presenting approximately 30 peptides from the Gag, Nef and Pol proteins. In contrast, the alleles possessed by people with higher viral loads could only present around 10 such antigens, a highly significant difference. Ranasinghe noted that this work represents the first evidence that HLA DRB1 alleles influence viral load at the population level. —RJ

by vaccines, but Vinuesa noted that research into the impact of different vaccine approaches on Tfh cell and Tfh/B cell interactions is still in its infancy. The finding that bNAbs are characterized by extensive somatic hypermutation—the genetic changes in B cells that occur during affinity maturation (see *Vaccines to Antibodies: Grow Up!*, IAVI Report, July-Aug. 2010)—suggests that a better understanding of Tfh cells could contribute significantly to HIV vaccine development.

There followed other intriguing presentations on Tfh cells. Madelene Lindqvist, a postdoctoral researcher in the laboratory of Ragon Institute immunologist Hendrik Streeck, presented the first data on Tfh cells ever collected from individuals with chronic HIV infection. Her study involved 16 individuals with untreated infections (a median CD4⁺ T-cell count of 444 and a viral load of 35,000 copies/ml), 10 on antiretroviral therapy (median CD4⁺ T-cell count of 573 and viral load of 49 copies/ml) and seven HIV-uninfected controls. Lindqvist first demonstrated that Tfh cells with the profile outlined by Vinuesa (displaying CXCR5 and PD-1, expressing Bcl-6 and producing IL-21) were present in lymph node samples from participants at a frequency that was 100-fold higher than that measured in peripheral blood.

Comparing the different study cohorts, Lindqvist found that Tfh cells were significantly expanded in untreated HIV infection compared to controls. Gag-specific Tfh cells were found to be a component of this expansion, as the magnitude of such responses was two-fold higher in untreated vs. treated infection. Tfh responses to gp120 were also measured, but were around five times lower than those to Gag. Additional analyses revealed correlations between Tfh expansion and the B-cell dysregulation that has been described in progressive HIV infection (*Nat. Rev. Immunol.* **9**, 235, 2009). Specifically, increased Tfh numbers in untreated HIV infection correlated with an increase in plasma cells and loss of memory B cells in lymph nodes, along with the hypersecretion of IgG antibodies (hypergammaglobulinemia). Lindqvist noted that B-cell dysregulation in HIV had been thought to be a consequence of compromised CD4⁺ T-cell help. Her data indicate, however, that it is driven by an abnormal expansion of Tfh cells.

Learning from the living

Researchers are learning more about what drives protective immune responses to HIV-like retroviruses by studying, in nonhuman primates, the effects of live attenuated vaccines (LAVs) made from weakened strains of simian immunodeficiency virus (SIV). Such experimental vaccines provide macaques with the most robust protection obtained against SIV. Although preventive LAVs against HIV are generally considered too risky for use in humans, the field has placed a high priority on understanding why precisely they work so well in experimental models and applying that information to improve the design of HIV vaccines.

Yoshinori Fukazawa, a postdoctoral researcher at the Oregon Health & Science University's Vaccine and Gene Therapy Institute, presented new data at CROI that described the strongest correlates of LAV protection obtained so far. Fukazawa's experiment evaluated five different LAVs with six macaques in each vaccine group and six unimmunized controls. Challenge was performed intravenously with SIVmac239 at week 50 after LAV inoculation. The extent of protection achieved was diverse, with only SIVmac239 Δ nef and SIVmac239 Δ 3 offering complete protection, which Fukazawa primarily defined as no—or only transient—replication of the challenge virus, and no depletion of mucosal CD4⁺ T cells.

Eleven different immunological parameters were assessed as potential correlates of immunity, including ADCC, neutralizing antibodies and SIV-specific T-cell responses (both CD4⁺ and CD8⁺) in blood, lymph nodes and lung. Highly significant correlations with protection were only observed for SIV-specific CD4⁺ and CD8⁺ T-cell responses measured by intracellular cytokine staining (ICS) in the lymph nodes ($p=0.0036$ and $p=0.0070$, respectively), after Bonferroni corrected for multiple comparisons. The T cells displayed an effector memory phenotype. Using an assay designed to measure the ability of SIV-specific CD8⁺ T cells to suppress viral replication in autologous infected CD4⁺ T cells, Fukazawa also found differences between protected and non-protected animals. But these were only statistically significant for SIV-specific CD8⁺ T cells sampled from lymph nodes, not from blood.

The subject of Tfh cells cropped up again in Fukazawa's talk, when he produced evidence suggesting that LAVs preferentially replicate in that population. Furthermore, the amount of LAV RNA in lymph nodes correlated significantly with the magnitude of the SIV-specific CD4⁺ and CD8⁺ T-cell responses at the site. Fukazawa concluded that ongoing LAV replication is probably essential to maintaining protective effector memory T-cell responses in lymph nodes. This suggests that persistent vaccine vectors will in all likelihood be required to induce and maintain this type of immunity in humans.

Molecular warfare

Researchers continue to study HIV's ancestral tree to better understand the emergence of viral defense mechanisms. The tussle between viruses and hostile host environments leads to an "evolutionary arms race" that has shaped the evolution of HIV.

It has, for one thing, spurred the selection of several HIV-1 proteins to counteract the effects of defensive host cell proteins—restriction factors—that curtail viral replication. The latest restriction factor identified is a cellular protein named SAMHD1, which limits the ability of HIV-1 to replicate in dendritic cells (DCs) and monocytes by depleting the nucleotide components the virus needs to assemble new virions (*Nat. Immunol.* 13, 223, 2012). Some members of the lentivirus family to which HIV belongs possess a protein, Vpx, which has the ability to degrade SAMHD1, allowing these viruses—including HIV-2 and its simian virus precursors—to infect dendritic cells and monocytes.

Olivier Fregoso, a postdoctoral fellow in the laboratory of Michael Emerman at the Fred Hutchinson Cancer Research Center in Seattle, described his efforts to ascertain whether the ability to degrade SAMHD1 is a function that HIV-1 has lost, or one that its viral relatives have gained. The question is not merely academic: understanding the emergence of adaptations can shed light on hidden vulnerabilities that emerge at various points in the viral life cycle.

Fregoso showed that the ability to degrade SAMHD1 was acquired initially by Vpr in a lineage of lentiviruses distinct from the one out of which HIV-1 evolved. The Vpr protein in this lineage subsequently evolved into Vpx, a protein specializing in the degradation of SAMHD1. HIV-1, it would appear, has not evolved to avoid infecting DCs and monocytes, as was suggested when SAMHD1 was first identified. Rather, Fregoso's group believes that HIV-1's greater pathogenicity compared to HIV-2 could be related to its *inability* to infect these cell types (*Cell Host Microbe* 11, 194, 2012).

Two restriction factors were discovered prior to SAMHD1. One was APOBEC3G, a cellular protein with antiretroviral activity that is degraded by the HIV-1 protein Vif, the other, tetherin, which limits viral replication by tethering virions to the cell and preventing their dissemination. Tetherin is counteracted by the HIV-1 protein Vpu. APOBEC3G, meanwhile, belongs to a family of APOBEC3 proteins (designated A, B C, DE, F, G and H). Emerman showed evidence that the selection of a version of APOBEC3DE with increased anti-

viral activity occurred in response to a virus that infected chimpanzees around 2.5 million years ago (*J. Virol.* 85, 11361, 2011). The human version lacks similar activity because the virus-driven change occurred after the evolutionary branches of humans and chimpanzees diverged.

Tetherin, too, provides a striking example of the impact of viral adaptation. In chimpanzee SIV (SIVcpz), the precursor to HIV-1, the antiviral effect of tetherin is blocked by the Nef protein. But the specific site on tetherin that SIVcpz Nef binds doesn't exist in the human version of the protein. HIV-1 group M, the strain primarily responsible for the worldwide pandemic, overcame this obstacle by adapting and deploying the Vpu protein to subvert tetherin. The far rarer HIV-1 group O did not; and in group N viruses the anti-tetherin activity of Vpu is far less consistent. HIV-1 group M's arms race against tetherin has, in a sense, helped shape human history (*J. Virol.* 84, 7124, 2010).

Sticking to PrEP

As it did last year, and the year before, ARV-based prevention dominated discussion at CROI. In particular, attendees sought to mine the implications of fresh data from a number of pre-exposure prophylaxis (PrEP) studies that suggest PrEP may be significantly less feasible in some high-risk populations than had been hoped.

"The reality doesn't necessarily match the vision," said Jared Baeten, a University of Washington associate professor of global health and a co-investigator in the Partners PrEP study, which found high efficacy in serodiscordant couples. "We have four completed PrEP trials that demonstrate efficacy, but we have two trials in women with high incidence where the entire study or individual arms have demonstrated futility."

One of them was FEM-PrEP, which enrolled nearly 2,000 high-risk heterosexual women from Kenya, South Africa, and Tanzania. The study was discontinued in March, 2011, after a data safety monitoring board determined it was unlikely to establish whether or not daily oral administration of Truvada—a combination of the ARVs tenofovir (TDF) and emtricitabine (FTC)—is effective in reducing HIV acquisition (see *Vaccine Briefs, IAVI Report*, Mar.-Apr. 2011).

Lut Van Damme, the trial's principal investigator, presented a final analysis of the trial data that suggests inadequate adherence to the prescribed drug regimen may have undermined the trial, which tallied 33 infections in its Truvada arm and 35 in its placebo arm. Analyses of the blood

Continued on page 21

TAPPING the Sanguine Humor

Broadly neutralizing antibodies stole the show at Keystone

By Regina McEnergy

A vaccine that elicits broadly neutralizing antibodies (bNAbs) to HIV has long seemed at least as elusive as the Holy Grail. But as more and more such antibodies are isolated from HIV-infected volunteers—revealing rare vulnerabilities on HIV—researchers are increasingly hopeful of coaxing that coveted humoral response.

Some 400 attendees at the Keystone Symposium, which paired the annual HIV Vaccines meeting with the Viral Immunity and Host Gene Influence meeting, got an update on the quest to make such vaccines. A number of talks at the conference, held March 21-26, illuminated how structural and computational biology are being applied to reverse-engineer immunogens that might induce bNAbs. Others described how next-generation DNA sequencing technologies are unraveling the genetic origins and evolution of those antibodies.

Researchers are also making significant progress toward solving the unliganded structure of the Envelope trimer, the lack of which has long hampered immunogen design (see *A Slew of Science in Seattle*, this issue). Yet the insights gleaned from such breakthroughs, and from other lines of inquiry into the ontogeny of the B cell response to HIV, have also exposed just how hard it will be to elicit appropriate antibodies. Indeed, Gary Nabel, director of the Vaccine Research Center (VRC) at the US National Institute of Allergy and Infectious Diseases (NIAID), was moved to quote Sir Winston Churchill while describing progress in that endeavor: “This is not the end, it is not even the beginning of the end. But it is perhaps the end of the beginning.”

If so, the start hasn't been half bad. The field, as Nabel noted, now has access to more than 100 bNAbs against HIV, many of which have not yet

been described in the literature. Structural, genetic and biochemical analyses of these exquisitely precise molecules are providing valuable clues to the structure-assisted design of vaccines.

The sole target for those antibodies, the HIV envelope trimer, is a wonder of evolutionary refinement. The few functional trimers scattered over HIV's surface, for example, encode a number of immunodominant decoys for antibodies, most notably in the variable loop domains. These provoke a vigorous but generally useless antibody barrage that drowns out potentially neutralizing responses. Beyond that, the spike is covered with a thick fur of complex carbohydrates—often called the “glycan shield”—that largely resemble the complex sugars found on human cells, and are thus often ignored by the immune system. The bulky sugar chains also restrict antibody access to underlying peptide epitopes that might otherwise elicit neutralizing responses.

Nabel and his colleagues at the VRC have sought to pierce these defenses with their immunogens. They have covered up regions on the envelope that are highly immunogenic but essentially serve as decoys, and have removed glycans that hinder access to conserved epitopes. They have rearranged glycans on the surface of the protein, and altered some known immunodominant regions by modifying the variable loops, so as to bias the response in favor of neutralizing antibodies. Yet the responses induced thus far in mice, rabbits and nonhuman primates (NHPs) suggest they have a ways to go.

Not, however, for want of creativity. One immunogen developed at the VRC contains a re-engineered monomeric gp120 stripped of its inner domain, an excision intended to improve antibody responses to the CD4 binding site. Unfortunately,

the excision destabilized the gp120 protein. So the VRC team secured the required structure by introducing disulfide bonds via directed mutagenesis. They then studded the surface of a Chikungunya virus-like particle with an array of these stabilized proteins—the immune system recognizes and responds better to large protein particles—and tested their immunogen in rabbits (see *A Bangkok Surprise, IAVI Report*, Sep.-Oct. 2011).

Nabel and his colleagues at the VRC have found that the scaffolded subunit immunogen, whether administered alone or in a prime-boost regimen, induces antibodies in rabbits that target the CD4 binding site. “The bad news,” said Nabel, “is that the antibodies are hitting mostly tier 1 viruses.” These are the most sensitive of HIV variants, and are not representative of those that circulate naturally.

One reason for the limited neutralization, said Nabel, could be that the addition of glycans to the resurfaced core of gp120—done to block immunodominant regions and refocus the response on the CD4 binding site—was not extensive enough. A number of antibodies that are not broadly neutralizing recognize regions of gp120 similar to those bound by b12 and b13. The target areas of partially masked immunogens might therefore have to be further constrained to elicit bNAbs like b12 and b13, which approach the CD4 binding site from very specific angles.

While much immunogen design has thus far focused on that highly conserved site, the discovery last year of a new family of bNAbs—some of them extremely potent—that mostly appear to be glycan-dependent has given researchers a new target for immunogen design (*Nature* 477, 466, 2011). The antibody PGT128 is among the most potent in this family of so-called PGT antibodies, said Dennis Burton, a professor of immunology and microbial science and the director of IAVI’s Neutralizing Antibody Center at The Scripps Research Institute. It is capable of neutralizing 70% of a panel of 162 HIV pseudoviruses that represent all HIV subtypes currently in circulation. Burton noted that PGTs 135-137, if not as broadly neutralizing as PGT128, are still notably potent. Glycans at positions 332 and 392 on the trimer appear to be critical to their binding, said Burton. But a crystal structure of PGT135 bound to the gp120 core shows that it also makes contact with three other glycans.

“For the CD4 binding site, it’s clear you have to have quite a precise angle of approach,” said Burton. “This does not seem to apply to the glycan shield.” An antibody that approaches the CD4

binding site reaches out and “kisses” its target, he said. Glycan-dependent antibodies, on the other hand, only lightly embrace their sugar targets so as to make firmer contact with the peptide.

A base approach to neutralization

Some bNAbs—such as 4E10 and Z13—disable HIV by latching onto the very bottom of the HIV spike, a region of the trimer known to scientists as the membrane-proximal external region (MPER). Jinghe Huang, a researcher in the laboratory of Mark Connors, chief of the HIV-specific immunity section at NIAID, detailed the isolation of a new bNAb that binds the MPER stalk in a rather unusual way.

This new bNAb, 10E8, appears to target an epitope that overlaps the one bound by 4E10 and Z13. 10E8 also appears to bind two additional amino acids within MPER, both of which are highly conserved residues. MPER antibodies are known to bind phospholipid, are autoreactive, and bind poorly to envelope prior to CD4 docking. However, Huang noted that the 10E8 antibody doesn’t bind phospholipid and does not appear to be autoreactive. It binds envelope on the surface of infected cells, and that binding is more resistant to washing of virions compared to other MPER antibodies, meaning that its epitope is accessible on the unliganded HIV Envelope trimer.

The neutralization potency and breadth of 10E8 was tested against a panel of 181 HIV pseudoviruses in parallel with five other bNAbs: 4E10, 2F5, VRC01, PG9 and PG16. At low concentrations, 10E8 could neutralize 72% of the tested viruses, similar to VRC01 (75%) but considerably more potent than 4E10 (37%) and 2F5 (16%). When infused into rhesus macaques, 10E8 provided 100% protection from mucosal challenge with simian human immunodeficiency virus (SHIV). “Discovery of 10E8 certainly puts gp41 back on the table in terms of vaccine targets,” said Huang.

A shocking strategy

HIV immunogens are hard enough to make, but even the most promising ones typically elicit sera that neutralize only the most sensitive viruses in established assays. Richard Wyatt, director of viral immunology in IAVI’s Neutralizing Antibody Center, explored whether a judiciously selected immunogen in the DNA prime—delivered via electroporation—and a boost using an adjuvanted, soluble trimer could improve outcomes. His team assessed three prime-boost regimens in rhesus macaques. Most of the primes

[RV144: TAKE 2, SORT OF...]

The central unanswered question raised by the RV144 trial is how on earth the prime-boost combination it tested actually worked. After all, when evaluated separately, neither showed even a hint of possible efficacy. Together, however, they provided 31.2% protection against HIV.

Researchers have been attacking this troubling conundrum from a variety of angles, not least by immunizing nonhuman primates with simian immunodeficiency virus (SIV) vaccine regimens that mimic those used in the RV144 trial. At Keystone, Poonam Pegu, a postdoc in the laboratory of Genoveffa Franchini, chief of the animal models and retroviral vaccine section at the US National Institutes of Health's National Cancer Institute, presented results of a pilot study of 21 Indian rhesus macaques, half of which were vaccinated with an ALVAC-SIV/SIVgp120 prime-boost combination that followed the same dosing schedule as RV144. The animals were subsequently challenged rectally with a low-dose of the highly pathogenic SIVmac251.

The animals were challenged with SIV once a week for five weeks. After five weeks, all of the controls had become infected, but in the vaccination arm of the study, about a third of the animals remained protected—a rate almost identical to that achieved in the RV144 trial. The viral load levels and CD4⁺ T-cell counts were no different in SIV+ animals, whether they had received the vaccine or not. The vaccinated animals protected from infection also had equivalent T-cell responses. But the antibodies they generated against gp120 bound with higher avidity than those of the unprotected animals.

Rectal challenges were stopped after five weeks because the unvaccinated animals all became infected by that time point. “We could have continued the challenge, but statistically we wouldn't have gained any power,” said Franchini. She added that the primary purpose of this pilot study was to see whether the specific dilution of the challenge virus was able to transmit just a few SIV variants. A larger study that will include 75 animals is planned for August to try and identify a correlate of protection. —RM

consisted of a DNA plasmid encoding cell-surface anchored, cytoplasmic-tail deleted gp160_{JR-FL}, the externally exposed part of a viral Envelope complex from a strain of HIV that is particularly difficult to neutralize. However, two of the 12 animals received DNA encoding the soluble gp140 foldon as a prime.

Wyatt's team electroporated the DNA plasmid to boost its uptake, administering it at 0, 1 and 2 months. Some of the priming constructs were codon-optimized to improve protein expression. The boost was a soluble gp140_{JR-FL} trimer with a C-terminal tag, or foldon, to improve stability. It was formulated with an adjuvant of carbohydrate polymers derived from yeast and administered at months 5 and 7.

Wyatt and his team found that DNA priming after the third injection achieved reasonable levels of binding antibody titers. However, “the big question is not [whether we get] binding antibodies but, do we get neutralization?” said Wyatt.

To find out, he and his team conducted a standard neutralization assay using TZM-bl, a HeLa cell derivative engineered to be highly susceptible to HIV. Serum collected after the boost neutralized the usual tier 1 viruses, and DNA priming didn't appear to contribute much to the scale of antibody neutralization. The DNA prime and a protein boost were required for tier 2 neutralization, and only three tier 2 viruses were neutralized. But Wyatt said studies using an A3R5 cell line—derived from CEM human lymphoblastoid cells—that is 10 times more sensitive than the TZM-bl assay, found substantial neutralization of the more resistant tier 2 viruses. In those assays, the DNA prime alone or with a protein boost elicited tier 2 neutralization. For most sera, all eight clade B and C tier 2 viruses were neutralized, but with decreasing potency on the more resistant isolates. (The A3R5 assay has been developed by David Montefiori, director of the Laboratory for AIDS Vaccine Research and Development at Duke University.)

Wyatt said his team now plans to repeat the experiment in a larger group of macaques to better quantify the results. “We also want to make the DNA prime better by increasing immunogenicity of the DNA candidate, and we want to generate trimers that preferentially present the neutralizing determinants, and not so much the non-neutralizing determinants.”

Orchestrating the right response

Structure-based vaccine design is often described as a sort of molecular sculpting. But Nancy Haigwood, director of the Oregon

National Primate Research Center at the Oregon Health and Science University, thinks it should instead resemble a symphony. “Viral vaccines that have worked in the past,” she explained, “have been symphonic in nature because they generate all types of immune responses.” Current approaches to HIV vaccine design are, in her view, more analogous to the work of musical soloists or, at best, chamber groups. “What we really need is a symphony: rich, iterative and sustained.”

In pursuit of that orchestral complexity, Haigwood's lab is assessing the antibody responses elicited by immunogens that contain multiple Envelope variants from a viral quasispecies population—the genetic HIV variants that exist within a single HIV-infected individual. Her strategy derives from the observation that B cells are programmed during affinity maturation to develop bNAbs through exposure to a series of Envs encoded by variants of viral quasispecies. Two years ago, Haigwood and colleagues used clones of native, longitudinal sequential Env variants that arose over years of infection to elicit bNAbs. The study tested three different immunization strategies in rabbits and found that a sequential vaccine approach using variants from a viral quasispecies population best replicated features of the humoral immune response in the subject from which these Envs were cloned (*J. Virol.* 85, 5262, 2011).

Haigwood and colleagues have since taken this approach one step further. They have exposed rabbits to a collection of Env variants representing the viral quasispecies of a single HIV-infected elite neutralizer who developed bNAbs relatively quickly—within two years of infection—with the breadth of the response further increasing over three years.

The researchers cloned fifty full-length functional Envelopes by single genome amplification at nine longitudinal timepoints, selecting 18 of them as vaccine candidates. The immunogens, which consisted of gp160 DNA and gp140 trimeric protein, were co-administered to rabbits using four different immunization strategies. Neutralizing antibodies were detected at six weeks, after only two immunizations, and increased after additional immunizations. Rabbits immunized with sequences that were representatives of Envelopes present at the time of first evidence of bNAb development achieved the highest neutralization potency of the four groups. This suggests that it might be possible to educate an immune system that is naïve to HIV by

methodically presenting it with immunogens derived from HIV quasispecies known to be present preceding the development of bNAbs.

The roots of the right response

Scientists have found that approximately 25% of HIV-infected individuals develop antibody responses that can neutralize a diverse array of primary viruses. But only a small percentage of this select group develops neutralizing responses that can be called both very broad and potent (*J. Virol.* 83, 188, 2009; *J. Virol.* 83, 7337, 2009). Yet, while such antibodies are more common than previously believed, much remains unknown about how they evolve in response to HIV infection.

It isn't clear, for example, when exactly bNAbs first turn up in HIV-infected individuals. This question isn't easily answered because most serum samples from which bNAbs are isolated came from individuals who had been living with the virus for years before they offered their blood up to science. But some rare cohorts of folks followed from the earliest stages of infection do exist, and two research groups separately parsed

sera from such volunteers to trace the evolution of bNAbs.

A research effort led by the University of Amsterdam had previously established that approximately 33% of HIV-infected enrollees in the Amsterdam Cohort had cross-reactive neutralizing activity (CrNA) in sera approximately 35 months following sero-conversion. It has also found that evidence of neutralizing activity does not correlate with slower disease progression (*J. Infect. Dis.* 201, 1045, 2010).

In their latest study, the Dutch team combed the sera of six HIV-infected men who have sex with men (MSM) previously enrolled in the Amsterdam Cohort Studies on HIV and AIDS, which began in 1984. The clade B HIV-infected men in this sub-analysis, who all joined the cohort in the late 1980s, had the most potent CrNA at 35 months after seroconversion. One of them was an elite neutralizer, which is to say his serum neutralized five pseudoviruses out of a panel of six pseudoviruses that have been statistically screened to predict neutralization breadth (*J. Virol.* 83, 7337, 2009). For their study, researchers obtained serum samples at three

Help from the Relatives

Attendees at Keystone also got an update on the first clinical study of a chimpanzee adenoviral vector—ChAdV-63—bearing a universal HIV-1 immunogen. The hope is that such vectors will provide an alternative to human Ad vectors, which as a class have had a bit of a rough ride as potential vehicles for HIV immunogens.

Most famously, analyses of the Phase IIb STEP trial indicate that the MRKAd5 viral vector vaccine candidate actually led to an increased risk of HIV acquisition in a subgroup of volunteers who were uncircumcised and had pre-existing adenovirus serotype 5 (Ad5) antibody immunity. Further, such pre-existing immunity dampens HIV-specific cellular immune responses to MRKAd5. As a consequence, a few research groups have been designing HIV vaccines built on vectors derived from human adenoviruses that are less common worldwide, such as Ad26 and Ad35 (see *Adenovirus Vectors: Promise and Possible Pitfalls*, *IAVI Report*, Jan.-Feb. 2012).

Others are looking at chimp Ad vectors as alternatives. A recent study named ChAdV-63 as being one of several such vectors that have an "immunological potency equivalent" to human Ad5 (see *Sci. Transl. Med.* 4, 115ra2, 2012). Tomáš Hanke, professor of immunology at the University of Oxford, presented immunogenicity results at Keystone of a pair of prime-boost vaccine regimens evaluating combinations of a universal immunogen, delivered in a modified vaccinia virus Ankara (MVA) vector, a DNA plasmid and a non-replicating ChAdV-63 vector. The HIVconsv universal immunogen is a chimeric protein consisting of

the 14 most conserved regions from clades A-D, with no more than a 6% variation between clades.

The ongoing trial enrolled 32 volunteers from the UK. In the safety arm, two volunteers received one shot of the low-dose ChAdV63.HIVconsv vaccine candidate. In the second arm, eight individuals were given the ChAdV63.HIVconsv candidate followed by the MVA.HIVconsv candidate at week 8. In the third arm, eight volunteers received the DNA.HIVconsv at weeks 0, 4 and 8, followed by ChAdV63.HIVconsv at week 12 and MVA.HIVconsv at week 20. And in the fourth arm, eight volunteers received the DNA.HIVconsv vaccine at weeks 0, 4 and 8 followed by MVA.HIVconsv at week 12 and ChAdV63.HIVconsv at week 16. In each of the last three arms, two additional volunteers received a placebo vaccine.

Preliminary data show that the heterologous prime-boost regimens were well-tolerated and highly immunogenic. Vaccine-induced T-cell frequencies among volunteers who received the ChAdV-63/MVA combination reached a median of 5150 SFU/10⁶ PMBCs—with a range of 1,475 to 16,495—as measured by IFN-γ Elispot. That response was specific for epitopes that are dominant when induced by the conserved vaccine but subdominant during natural infection, when they are overcome by responses to variable regions. The responses were composed of both CD4⁺ and CD8⁺ subtypes. DNA prime pushed these frequencies to a median of 7023 SFU/10⁶ PMBCs. By comparison, vaccine-induced T-cell frequencies in the STEP trial ranged from 136-686 SFU/10⁶. —RM

monthly intervals in the first year after seroconversion and at multiple intervals thereafter, and tested the sera for neutralizing activity.

Because it is too difficult and expensive for researchers to identify specific neutralizing antibodies in individuals, researchers used CrNA as a measure of bNAb production, said Hanneke Schuitemaker, who led the study before joining the Dutch-based Crucell last fall as its senior vice president for viral vaccine discovery and early development. Schuitemaker presented recently published data at Keystone that showed five of the men had developed CrNA 20-35 months following seroconversion. CrNA activity peaked in the men at 35 months, revealing the long time-frame in which such antibodies mature. The elite neutralizer, by contrast, showed signs of CrNA just 10 months after seroconversion (*J. Virol.* 86, 2045, 2012).

Researchers analyzed peripheral blood mononuclear cells (PBMCs) from the same six individuals with potent CrNA as well as PBMCs from three HIV-infected men who lacked CrNA to examine autologous clonal virus variants that appear over the course of infection. They did so because some quality of the transmitted founder virus that establishes infection is thought to influence an individual's ability to mount a cross-reactive neutralizing response. In all of the men, CrNA coincided with neutralizing activity against autologous strains that were isolated less than 12 months after seroconversion, while viruses from later points had already escaped autologous neutralizing activity.

The researchers are, of course, interested in learning more about the elite neutralizer. Schuitemaker said epitope mapping continues, but that new evidence contradicts the indications of preliminary findings that pointed to an epitope that includes the N322 position on the spike, associated with a glycan epitope. In collaboration with Lynn Morris, chief specialist scientist and head of the AIDS unit at the National Institute for Communicable Diseases in Johannesburg, Schuitemaker's team found that the serum neutralized a mutant virus that lacks N332. Further, mutations at the N332 position did not contribute to viral escape.

The rapid development of glycan-specific antibodies against position 332 of Env gp120 has previously been shown in monkeys (*Proc. Natl. Acad. Sci.* 108, 20125, 2011). "If we could have confirmed this epitope specificity in our elite neutralizer, it would have provided proof of concept for rapid development of this antibody specificity in humans," said Schuitemaker.

Out of Africa

A cohort of HIV-infected heterosexual men and women from Kenya, Rwanda, Zambia, South Africa and Uganda is providing its own clues on bNAb development. It derives from IAVI's Protocol C cohort, a longitudinal study of more than 600 donors that has been evaluating immunological and virological markers from the earliest stages of infection.

Elise Landais, a research associate at the IAVI Neutralizing Antibody Center at The Scripps Research Institute, presented neutralization data at Keystone on 328 of the 611 enrollees in Protocol C. At year two, only two of the individuals in this subgroup had developed broadly neutralizing activity, as defined above. By years three and four, the percentage of enrollees who possessed such activity had increased to 9% and 19%, respectively.

It appears, Landais said, that the development of cross-clade neutralizing activity is a gradual process. By 24-30 months, over 20% of enrollees were already able to neutralize three to four of the pseudoviruses—a capability that she considered indicative of moderate broadly neutralizing activity. "We know from previous studies that you won't find bNAbs early," said Landais, "so the point of this longitudinal study was to find out exactly when they develop. It was interesting to us that there seemed to be this intermediate step of moderate breadth. Maybe there is some kind of learning process for the antibody, that it evolves later on to neutralize other strains."

Once achieved, neutralization breadth appeared to be relatively stable over time, said Landais, though the potency of the neutralization was relatively low. "We are now wondering if breadth develops first and potency later," said Landais. "This is the type of question we want to answer."

The study also found that serum antibodies in six of 21 top neutralizers were sensitive to a mutation at the N332 glycan at the base of the V2 loop, one of the sites commonly targeted by broadly neutralizing antibodies. Landais and her colleagues now plan to sort antibodies from three or four of the most interesting samples collected from this cohort to see if they are similar to PGT123 or PGT128—a bNAb that engages glycans attached to N332 or N301. Some individuals also appear to possess PG9-like neutralizing activity in their serum; PG9 targets an epitope exactly where the V1 and V2 loops connect. "We are going to try and isolate antibodies of known specificities and retrace the history of their development, which is essential in order to make a vaccine that mimics the virus," she said. ■

Stalking HIV's SLEEPER CELLS

This year's HIV Pathogenesis meeting focused on latent HIV reservoirs—and how they might be eradicated

By Andreas von Bubnoff

Many scientists worry that as HIV research has become increasingly specialized, it appears more and more to be enclosing itself in an intellectual silo. This trend, they fear, hampers the exchange of valuable information with related areas of biological research and, if left unaddressed, could ultimately stunt the creativity and productivity of the field as a whole. To address such concerns, the organizers of the Keystone meeting on HIV Pathogenesis have in recent years invited colleagues immersed in other areas of inquiry to their annual conference.

This year, the gathering—convened in Whistler, British Columbia, from March 26 to March 31—was held jointly with a meeting on Virus Entry, Replication and Pathogenesis that covered general virology. “My hope is that everybody exchanges ideas [and] we get out of our silos,” said Michael Farzan, a virologist at Harvard Medical School and one of the organizers of the track that covered general virology.

This is not to say that HIV got short shrift at the meeting. Sessions dedicated to HIV covered everything from the mechanisms of elite HIV control to new strategies for blocking HIV's entry into its target cells. But, more than anything else, researchers at Keystone were treated to the latest findings in cure research, most notably those related to the exploration and eradication of HIV reservoirs.

The T cell as Trojan horse

The depletion of HIV reservoirs is all but the ultimate prize of cure research. In other words, it's a pretty tall order. Even those who have been on highly active antiretroviral therapy (HAART) for years and have suppressed HIV to undetectable levels in their blood, retain these reservoirs of chronic infection. The reservoirs consist mostly of CD4⁺ T cells that carry one or more copies of HIV

in their chromosomes. These latently infected cells don't ordinarily produce virus particles—until, that is, HAART is interrupted.

Another possible repository of latent HIV is in macrophages, although the *in vivo* relevance of this reservoir remains unclear. HIV researchers long suspected that it isn't established very soon after HIV transmission, said Quentin Sattentau, a professor of immunology at the University of Oxford. This is because macrophages aren't easily infected by free virus—not even by transmitted founder virus, which is the first and in many cases the only transmitted virus that generates productive infection. Sattentau, however, presented research at Keystone that calls that belief into question. His studies suggest that macrophages can, in fact, be efficiently infected through direct contact with infected CD4⁺ T cells, establishing a latent reservoir much earlier and more efficiently than previously suspected.

Sattentau and his colleagues isolated primary CD4⁺ T cells from volunteers, infected them with HIV, and mixed the cells with macrophages from the same volunteers. Typically, when HIV-infected cells contact uninfected ones, they form virological synapses—structures through which the virus buds and migrates directly into the uninfected cell. But Sattentau detected an entirely different path to infection in his experiments: the macrophages in his mixtures ingested the infected CD4⁺ T cells by phagocytosis. This ordinarily results in degradation or digestion of the ingested cell. But electron micrographs taken by Sattentau and colleagues revealed that virus-infected cells taken in by macrophages through phagocytosis were not rapidly degraded but, instead, released their virus into a subcellular compartment.

The researchers also found that three days after a one to two hour contact with infected T cells,

1%-10% of macrophages had HIV in their cytoplasm. This was not the case when macrophages were incubated with free HIV particles from the same number of CD4⁺ T cells. Sattentau concluded that the phagocytosis of infected T cells represents a new mechanism by which macrophages can get infected. This, he pointed out, doesn't mean that macrophages are never infected by free virus, but that such mechanisms of infection are probably less efficient.

Sattentau further reported that transmitted founder viruses can also infect macrophages via phagocytosis of infected CD4⁺ T cells. This suggests that the macrophage reservoir may be established far earlier than previously thought. Because it is the job of macrophages to ingest and digest dead and dying cells, Sattentau said, they are attracted to T cells that are dying from HIV infection. That likely happens on a large scale just days after infection, as soon as viral proliferation takes off in gut-associated lymphoid tissue (GALT).

The benefit of a nip in the bud

Still, most latent HIV is believed to reside in resting CD4⁺ T cells, and depleting such reservoirs might improve the prognoses of HIV infection. Mathias Lichterfeld of Massachusetts General Hospital in Boston reported data that support this hypothesis. He and his team found that people who start HAART during acute infection have HIV DNA levels in their latent CD4⁺ T-cell reservoir similar to that of elite controllers. Those levels are, moreover, about 100 times lower than those observed in people who start treatment only in the chronic phase of HIV infection.

Lichterfeld and his colleagues drew these conclusions from studies measuring the levels of HIV DNA in CD4⁺ T cells from nine HIV-infected people. All of them had been on HAART for about 10 years, but three were put on the regimen before they had HIV antibodies in their blood (which typically occurs two to four weeks after infection), while six started the treatment later than that. The HIV DNA detected probably included defective virus, but Lichterfeld said the amount of replication-competent viral DNA was also a lot lower in those who started treatment early. He and his team further found that the HIV-infected individuals who started HAART early had a microRNA expression profile—used as a marker of disease activity—similar to that observed in HIV negative controls, suggesting that they were healthier than those who started HAART late.

“The data suggest that you benefit from early treatment initiation,” Lichterfeld argued, “because it does reduce the amount of reservoir

that you have in your system. If treatment options become available that target the reservoir, it would probably be of benefit if you have a low amount of reservoir to start with.”

Replicating with HAART?

Draining HIV reservoirs in people on HAART requires not only the eradication of latently infected cells but an end to their replenishment as well. Researchers at Keystone presented conflicting data on whether residual HIV replication—the kind that would replenish latent cell reservoirs—occurs in people on HAART who have no detectable HIV in their blood.

Sarah Palmer, of the Karolinska Institute in Sweden, and her colleagues found no evidence of viral evolution in such people. That, she argued, suggests an absence of HIV replication—at least in the tissues examined.

Palmer and her colleagues determined the RNA sequence of single HIV particles in plasma taken from eight individuals just days before they started HAART. (Five of them started HAART during acute infection, and three during chronic infection.) They then compared these with sequences of single integrated proviral HIV DNAs in CD4⁺ T cells from samples of the same patients' peripheral blood and GALT taken between four and 12 years later.

While individuals who started treatment later had a more diverse set of unique HIV sequences, none of the volunteers showed any difference in the kinds of sequences found in the samples taken shortly before they started HAART and years later. This indicates that there is no sequence evolution in peripheral blood and GALT in people on HAART and, to Palmer, suggests that there is little or no residual viral replication. Palmer also said that most CD4⁺ T cells in these tissues only harbored one copy of integrated HIV DNA. This, she noted, is good news for HIV eradication efforts. “If you have to purge your reservoirs, you don't have multiple HIV molecules that have to be purged,” she said. “There is not a large amount of replication that continues to reseed the reservoir. [So] hopefully, once we purge this reservoir, it's not being reseeded as long as you stay on HAART.”

Not everyone agrees. Tae-Wook Chun, an associate scientist at the National Institute of Allergy and Infectious Diseases (NIAID), said that HIV might replicate at such a low level that many more sequences in tissue sites might have to be determined to be sure it isn't evolving. “It might take several years to see meaningful viral evolution,” Chun said.

Mario Stevenson of the University of Miami

added that the HIV sequences Palmer looked at in tissues came from integrated provirus and could therefore have been generated from HIV that replicated a long time ago, before it integrated into the cell's genomes. A better way to detect recent HIV replication, he suggested, is to study nonintegrated HIV DNA, which only stays in cells a few days after HIV replication and then gets degraded. In previous studies, Stevenson and his colleagues found nonintegrated DNA in the GALT and lymph nodes of about a third of a group of people who had been on HAART for three and a half years on average (*Nat. Med.* 16, 460, 2010).

Stevenson said this probably indicates that HIV is replicating in these supposedly latently infected tissues. He cautioned, however, that the presence of nonintegrated DNA could be the result of only one cycle of infection, where a cell got infected but didn't pass the virus on to other cells. To distinguish between the two possibilities, Stevenson and his colleagues are currently sequencing single, unintegrated HIV DNAs in the GALT and lymph nodes collected from HAART patients to see if the virus is evolving. Such sequence evolution would suggest multiple rounds of HIV replication.

Stevenson also reported results from a study in which biopsies of GALT and lymph node tissues were taken from patients at zero, one, three and six months after they started HAART. The tissues were then assayed for viral RNA, nonintegrated viral DNA and intracellular drug levels in CD4⁺ T cells. Virus was undetectable in the plasma of the patients one month after HAART was started, but still detectable in GALT and lymph nodes; six months into therapy, most of the 15 individuals studied so far actually had more viral RNA and nonintegrated viral DNA in GALT and lymph node tissue than they had before they started treatment. The lymph nodes, which had the most viral transcripts, actually had the lowest intracellular drug levels in their CD4⁺ T cells, suggesting that drug concentrations there were insufficient to suppress viral replication. "Some drugs in lymph node are undetectable," Stevenson observed.

One possible explanation for this, said Stevenson, is that the cells in lymph nodes are more activated than cells in blood, and more activated cells are more likely to actively export drugs. "If that's the case, then we could come up with strategies to inhibit the drug exporters," he said. In any case, he added, his results suggest that the latent reservoir in people on HAART is constantly replenished by a low level of ongoing replication in some tissues. "I don't think we can be arrogant enough to say that the viral reservoir is simply viral latency, and noth-

ing else is going on," Stevenson said. "If we are to eradicate the reservoir, the first thing we are going to have to do is take care of replenishment."

HAART in simian models

Studies of the latent HIV reservoir in HAART patients are difficult to conduct because they involve repeated biopsies. There is thus an urgent need for animal models that mimic HAART. Researchers at Keystone reported that they may have come up with such models.

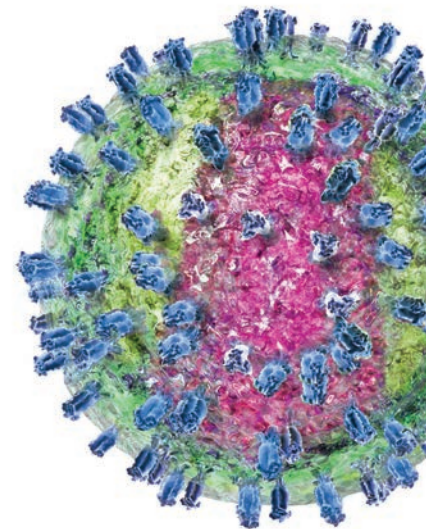
Binhua Ling from the Tulane National Primate Research Center accomplished the feat in Chinese rhesus macaques infected with the mac239 strain of simian immunodeficiency virus (SIV). All four of the chronically infected animals she studied had undetectable levels of SIV—defined as less than 30 copies per ml of plasma—for two months in response to treatment with a combination of three antiretroviral medicines (ARVs). (One, however, did have detectable viral load at one time point.)

Jeffrey Lifson of the AIDS and Cancer Virus Program at SAIC Frederick, Inc., Frederick National Laboratory, who collaborated with Ling on her study, reported that in a separate study, he and his colleagues achieved suppression of SIVmac239 viral load to undetectable levels in six Indian rhesus macaques with a combination of several ARVs. This was probably tougher to pull off, said Lifson, because SIVmac239 tends to replicate to higher levels in Indian rhesus macaques than in the Chinese variety.

Reactivating the reservoir

One currently favored strategy for eradicating viral reservoirs involves inducing HIV replication in latent cells, so that they die as a result of renewed virus replication or can be targeted by drug treatment or immune responses. One drug that researchers hope might roust HIV from its hiding places is the histone deacetylase inhibitor SAHA. Lifson, in fact, is currently testing that possibility in his Indian rhesus macaques.

SAHA, as it happens, is also being tested in humans. Recently, David M. Margolis of the University of North Carolina at Chapel Hill reported promising results from one of the first Phase I human trials examining this drug's potential as a rouser of latent HIV. He recently reported that, in a handful of HIV-infected individuals on HAART, a single dose of the drug leads to an increase in cell-associated HIV RNA expression (see *In Pursuit of a Cure*, *IAVI Report*, Jan.-Feb. 2012). At Keystone, Margolis shared further results that now included seven patients from the study. On average, SAHA treat-



[INSIGHTS INTO THE ELITE
CONTROL OF INFECTION]

One reason some people can suppress HIV even without HAART is that their CD8⁺ T cells are better able to kill HIV-infected cells. This is especially true of those who have certain MHC receptor variants that infected cells use to present HIV antigens to CD8⁺ T cells. Variants called HLA B27 and B57 have, for example, been associated to some degree with the ability to suppress viral load to very low levels.

Yet, oddly enough, most people who have those two variants don't display lower viral loads, suggesting that other factors contribute to the suppression of HIV in elite controllers who carry these genes. To identify those factors, a team led by Bruce Walker of the Ragon Institute studied 10 HIV-infected people, all of whom had the HLA B27 variant, but only half of whom were able to control their viral load.

The key difference between controllers and non-controllers, it turned out, wasn't in the types of epitopes presented to CD8⁺ T cells, Walker reported. In people with HLA B27, just one HIV Gag epitope called KK10 is presented to CD8⁺ T cells. A closer look at the KK10-specific CD8⁺ T cells did reveal, however, that in the elite controllers these cells were better able to kill HIV-infected cells and inhibit virus replication *in vitro* than in the non-controllers.

Because there was no difference in the antigen presenting cells between the two groups, the researchers reasoned that the secret of better control might reside on the other side of the equation: the T-cell receptors (TCRs) on CD8⁺ T cells that recognize the KK10 epitopes. And indeed, TCR gene sequences in KK10-specific CD8⁺ T cells revealed that controllers had TCR types that were also found in the CD8⁺ T cells that could better kill virus *in vitro*. By contrast, none of the TCR types found in the non-controllers were found in the more efficient CD8⁺ T cells.

Walker noted that knowledge of the TCR types induced by a vaccine might allow researchers to assess if a vaccine response is likely to be effective. He and his colleagues are already looking at which TCR types are induced in volunteers who received a vaccine candidate containing HIV Gag. —*AvB*

ment led to a roughly 5-fold increase of cell-associated HIV RNA detected in CD4⁺ T cells in the blood.

However, Chun reported that a 48-hour *ex vivo* SAHA treatment of resting, latently infected CD4⁺ T cells isolated from the blood of HIV-infected individuals on ART did not increase the number of free viruses (measured by counts of cell-free HIV RNA) produced by the cells, as compared to untreated cells.

Margolis said one possible reason for the difference between his observations and Chun's could be that 48 hours might be too long as a treatment. "Prolonged exposure to these drugs at this concentration [could] probably have nonspecific effects on the cell," he said, adding that 48 hours is much longer than it takes for one dose of the drug to be cleared out of the body. Chun, however, countered that the cells in his experiments were very viable, suggesting that his treatment wasn't too toxic, and that he got similar results after treating cells for less than 24 hours. Still, Margolis isn't convinced: Even if normal cells are not killed by SAHA *in vitro*, he said, such exposure may prove too toxic for use in the clinic. Chun, for his part, offered a different explanation for the discrepancy in their findings, noting that he assayed RNA levels of free HIV particles, whereas Margolis measured the levels of HIV RNA inside cells. It is thus possible, Chun argued, that SAHA induces an increase in HIV RNA transcription, but that this does not necessarily translate into an increase in HIV virions actually released from the cell.

A direct route to the cure?

The only person whose HIV infection is believed to have been cured is Timothy Brown, who underwent a number of anti-cancer treatments for acute myeloid leukemia before receiving a risky bone marrow transplant. The marrow donor was homozygous for the CCR5 Δ 32 allele, which abrogates expression of the HIV co-receptor CCR5 on cells, leaving carriers resistant to HIV infection (see *In Pursuit of a Cure, IAVI Report*, Jan.-Feb. 2012). It remains unclear exactly which part of Brown's complex cancer therapy cured him, and whether this feat can be replicated in others. Researchers are therefore interested in reproducing the Timothy Brown result.

John Mellors from the University of Pittsburgh noted that Brown had received an allogeneic bone marrow transplant, in which the replenished marrow comes from a different person. The donor cells (which have to be matched in at least seven of the eight MHC alleles) used in such transplants recognize the remaining host cells as foreign and kill them. This makes it more likely, said Mellors, that the

donor cells will fully replace the host's immune cells if introduced after whole body irradiation. But there is also a chance that the host cells will reject and kill the donor cells. In that case, the individual would die from leukemia because the remaining leukemic cells would outgrow the normal cells from the donor. This, Mellors explained, is partly why allogeneic stem cell transplants have a 25% mortality rate.

Mellors and his colleagues thus studied 10 HIV-infected individuals from three medical centers in the US who had received a less risky variation of the treatment: an autologous bone marrow transplant, in which the patient's immune cells are replaced with his or her own bone marrow cells. This approach has a mortality rate of less than 5%. Its disadvantage, though, is that some of the transplanted cells might carry integrated HIV DNA. "You can't sort cells for latent HIV," Mellors said, adding that this is probably one of the reasons why this approach has not cured any of the 10 HIV-infected individuals he studied. Another possible reason is that the approach doesn't result in the replacement of all host cells because the donor cells don't kill existing cells that they recognize as self.

Mellors and colleagues are therefore enrolling 15 HIV-infected individuals with leukemia or end stage refractory lymphoma, who need an allogeneic bone marrow transplant, into a clinical trial. They will also try to find donors that are homozygous for the CCR5 Δ 32 allele, as was Brown's.

If this results in a cure in at least some cases, it will prove that Timothy Brown's case was not just a fluke, and could help elucidate why he was cured. Whatever happens, though, the approach isn't likely to ever become routine, given its high risk. "It's just proof of principle, that's all," Mellors said. "It's got no practical significance whatsoever, none. This is heroic, and totally impractical."

Perhaps, but the Timothy Brown story has also inspired the formulation of more practical cure strategies. Paula Cannon of the University of Southern California reported that she and her colleagues are preparing a human trial that involves autologous bone marrow transplants. Before the cells are transplanted back to the patients, however, they will be treated with modified adenoviral vectors that carry the gene for an enzyme that knocks out the CCR5 gene. This typically results in destruction of the CCR5 gene in about 5% of the cells, she noted. But studies in humanized mice suggest that because the descendants of cells successfully modified this way are resistant to HIV, they have a competitive advantage once infused into an HIV-infected animal, and eventually become the dominant population of

immune cells (*Nat. Biotechnol.* 28, 839, 2010). Cannon said her team is preparing to apply for FDA approval to test the strategy in humans.

Another approach to curing HIV infection relies on the excision of integrated HIV DNA from cells that carry it. Jan Chemnitz from the Heinrich Pette Institute in Hamburg reported that he and his colleagues have made humanized mice that express Tre recombinase, an enzyme that can excise HIV DNA from the genomes of infected cells. To do so, the team transduced human CD4⁺ T cells or CD34⁺ hematopoietic stem cells with a lentiviral vector expressing Tre recombinase, and injected the genetically modified cells into humanized mice so that the cells could populate their immune system.

When they infected the mice with HIV-1, they found that the mice expressing the enzyme had significantly lower viral load and more CD4⁺ T cells than those that did not express it. Chemnitz said this is the first therapeutic approach that can remove proviral DNA of circulating wildtype HIV strains from the genome of an infected cell, and the first proof in an *in vivo* model that such an approach can attenuate HIV infection. Some animals were more or less cured, he said.

In its current form, the strategy does not excise HIV DNA from latently infected cells because HIV Tat (which is only present in productively infected cells) must be present to induce expression of the recombinase. But infusing patients with CD4⁺ or CD34⁺ cells that can express the recombinase should help increase the proportion of healthy immune cells that can easily get rid of HIV once they get infected. If combined with a treatment that reactivates the latent reservoir, this healthier immune system might be better able to kill infected as well as reactivated cells, Chemnitz suggested.

Another limitation is that the Tre recombinase used in the experiments can only excise integrated HIV DNA from an HIV clade A strain from Tanzania. The researchers are now designing a recombinase that they hope will excise many other HIV strains as well. Chemnitz plans a human trial using an enzyme with a broader specificity in about two years. He and his colleagues also hope to modify their strategy to target the latent HIV reservoir, developing lentiviral vectors that can be used to excise HIV DNA in latently infected cells even in the absence of HIV Tat.

Molecular decoys for HIV

Because the gp120 part of HIV's Envelope spike needs to bind both CD4 and CCR5 proteins to infect target cells, free-floating molecules that

effectively mimic these receptors could serve as decoys that inhibit the process. Unfortunately, efforts to use CD4 itself as a competitive inhibitor of infection have so far failed, said Farzan. But new approaches appear to hold more promise.

Farzan shared with the audience at Keystone the results of studies using a chimeric molecule to effect such inhibition. The molecule bears not only the part of the CD4 receptor that binds gp120, but also the portion of the CCR5 co-receptor that binds most tightly to gp120. That part, identified by Farzan's lab in 1999, consists of four sulfotyrosines (tyrosines that carry a sulfate group). Farzan and colleagues fused these sulfotyrosines to a modified version of CD4, called CD4 Ig, that consists of the two gp120-binding immunoglobulin domains of the CD4 receptor fused to the constant (Fc) domain of an antibody.

Farzan said that the resulting molecule has a 100% breadth of binding, and a potency similar to recently identified broadly neutralizing antibodies (bNAbs), such as VRC01. His chimeric protein neutralized all 22 HIV-1 strains tested, including the most evasive variants and strains that use the CXCR4 co-receptor to infect cells. It even neutralized three SIV strains and two HIV-2 strains, something no bNAb has yet accomplished. "That's a degree of breadth that's unprecedented," Farzan said.

He noted that because the molecule binds to both the CD4 and the CCR5 binding sites on gp120, HIV variants that mutate to escape it would in all likelihood also be much less efficient at infecting cells and replicating. Because it is a protein, Farzan said, the molecule may not be suitable for use in a topical microbicide gel formulation. Instead, he and his team plan to express it in muscle cells, by injecting adeno-associated virus (AAV) carrying a gene encoding the molecule into muscle cells. This gene transfer approach, developed by Philip Johnson of The Children's Hospital of Philadelphia, has already been shown to express truncated antibodies, including CD4 Ig, in rhesus macaques for almost two years, Farzan said, adding that such persistent expression protected the animals from challenge with an easily neutralized virus (*Nat. Med.* 15, 901, 2009).

Farzan hopes to use this AAV gene transfer approach in rhesus macaques to test whether it can protect against challenge with more robust viruses and drive down viral load in infected animals. The approach should be quite safe in humans, Farzan suggested, because it uses a modified AAV that does not integrate into the genome but stays in muscle cells—which don't divide—as episomal DNA that doesn't multiply. It therefore does not carry the risk of causing cancer. ■

Vaccine BRIEFS

WHO Updates Guidelines on ARV Treatment

The World Health Organization (WHO) released new treatment guidelines in April for low- and middle-income countries with generalized epidemics. The revised guidelines strongly recommend that HIV-infected individuals in serodiscordant relationships be offered antiretroviral (ARV) treatment when they have more than 350 CD4⁺ T cells per microliter of blood. The recommendations stem from compelling data obtained from recent studies of treatment as a mode of prevention, notably the HPTN052 trial involving 1,763 serodiscordant couples (see *Treatment is Prevention, IAVI Report*, July-Aug. 2011), which demonstrated the pronounced benefits of earlier ARV administration in reducing HIV transmission.

The new guidelines come in the wake of a statement by the WHO on the use of hormonal contraception that has drawn criticism from some public health experts for its vagueness (see *VAX* March 2012 *Spotlight* article, *A Hot Cup of CROI*).

The WHO had previously recommended that HIV-infected serodiscordant partners start treatment when their CD4⁺ T-cell counts were at or below 350/μl. The organization originally planned to announce the new guidelines for serodiscordant couples at last summer's International AIDS Society meeting in Rome, but held off to allow the dust to settle from an influx of

new data related to pre-exposure prophylaxis (PrEP) and couples counseling of injection drug users and men who have sex with men (MSM). However, the authors of the new guidelines ultimately omitted mention of any review of current data on the preventive benefits of antiretroviral therapy in populations other than heterosexual serodiscordant couples.

The 54-page report, posted on www.who.org, also recommends increased counseling and testing of serodiscordant couples in a manner that encourages mutual disclosure of HIV status in a controlled setting. The aim is to ease the psychological burden and curtail potential risks associated with receiving such information without professional support. Providing counseling and testing in such settings, it is hoped, will encourage joint decision making by the couple on matters related to safer sex practices, childbearing, and infant feeding. These settings include antenatal consultations, where the father and mother can learn together about their HIV status and their unborn child's risk of infection.

The WHO is currently reviewing and analyzing data from recent PrEP efficacy trials and expects to offer advice later this year to guide PrEP demonstration projects being conducted in MSM communities and among serodiscordant heterosexual couples. —*Regina McEnery*

Global HIV Vaccine Enterprise Appoints New Director

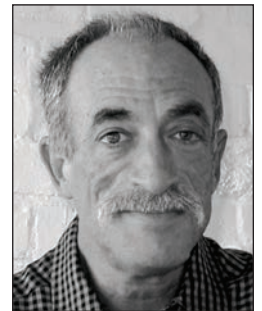
The Global HIV Vaccine Enterprise Secretariat, an organization that has, since its launch, struggled to find its niche, has picked a new leader. Bill Snow, who co-founded the HIV prevention advocacy group AVAC and is a respected voice among AIDS vaccine researchers, has been appointed its new director.

Snow takes the helm of a leaner organization, filling a vacancy created in June, when the inaugural director of the Enterprise Alan Bernstein departed after three and half years at the post. The Enterprise's seven-member board of directors, following an exhaustive review, released in October the details of a re-envisioned Enterprise Secretariat that will focus on three key priorities: coordination, collaboration and resource optimization. The main activities of the Enterprise will now include organizing the AIDS Vaccine Conference, holding an annual funders' forum later this year to optimize current resources and mobilize new funding, and convening relevant parties to address strategic issues (see *The Enterprise Changes Course, IAVI Report*, Sep.-Oct. 2011.)

"We have found the perfect person," says AVAC executive director Mitchell Warren and a member of the Enterprise's board. "There is no one who could provide better leadership or vision to the Enterprise than Bill."

Though not a scientist, Snow brings a certain "field cred" to the Enterprise that it has lacked since its inception, says Warren. Besides his longstanding involvement with AVAC, Snow also sits on various advisory committees including the AIDS Vaccine Research Subcommittee of the US National Institute of Allergy and Infectious Diseases, and the US National Institutes of Health's (NIH) Vaccine Research Center Scientific Advisory Working Group. He has also at one time or another contributed to the leadership of many HIV vaccine clinical trials groups.

Snow also served on the Enterprise's original council and as treasurer of its board when it received its first round of funding—largely from the Bill & Melinda Gates Foundation. His strong opinions about what the Enterprise could and should bring to the field convinced him to throw his hat in the ring. "I felt I knew the Enterprise as well as anyone except José," says Snow, referring to José Esparza, the senior advisor on vaccines for the Gates Foundation and the interim president of the Enterprise board. "The job



BILL SNOW

was too tempting to resist.” So tempting, in fact, that he and his longtime partner, interior designer Christian Nielsen, relocated from San Francisco to New York so Snow could take the job.

“We have no doubt that Bill’s leadership, intellect, passion and commitment will enable the Secretariat to help make this vision of the Enterprise a reality,” said Esparza, in a statement announcing Snow’s appointment.

The New York-based Secretariat, which consists of Snow and a staff of five, vacated its Union Square offices in March. It is now at 125 Broad St. in downtown Manhattan, where IAVI, one of its partners, is providing temporary workspace. “We and IAVI are considering it open-ended,” says Snow.

On the heels of the Enterprise’s change of leadership comes news that one of its bread and butter projects—the AIDS Vaccine Conference—will no longer be an annual event. At the International Microbicides Conference in Sydney this April, the two largest funders of HIV prevention research—the Gates Foundation and the NIH—jointly announced that, beginning in 2014, they no longer plan to fund single-themed conferences focused on microbicides, AIDS vaccines, or ARV-based prevention. Instead, they will fund a single biennial conference centered on all aspects of HIV prevention.

Continued from page 9

plasma collected during the trial from the 33 women in the Truvada arm who acquired HIV and about 99 matched uninfected controls found detectable levels of TDF in fewer than half of the samples. Notably, this contrasted with the 86% adherence rate suggested by the weekly pill counts—the number of pills dispensed minus those returned—as well as the claims of 95% of the volunteers who said they had taken the drug diligently. Van Damme noted that these data raise questions about the value of pill counts in measuring adherence, not to mention what became of the pills that were neither taken nor returned.

Van Damme also reported that there were 74 pregnancies (11%) in the Truvada group compared to 51 (7.5%) in the placebo arm of the study, which means that women in the Truvada group spent more time off PrEP because it had to be stopped due to safety concerns. Van Damme noted that pregnancy rates in the trial were highest among oral contraceptive users, which, she suggested, reflected a “problem with daily pill taking.”

Follow-up studies of the Partners PrEP trial underscore the importance of adherence as well. That study, which enrolled 4,758 heterosexual serodiscordant couples in Kenya and Uganda, revealed in 2011 that a daily dose of TDF reduced the risk of HIV infection by 62%, and a similar regimen of Truvada cut that risk by as much as 73% in the study population. Recent findings presented by Deborah Donnell, principal investigator of the HIV Prevention Trials Network and a statistician in the Partners PrEP study, indicate that individuals who remained HIV uninfected in the TDF and Truvada arms had detectable levels of the drug on 83% and 81% of their study visits, respectively. Drug levels were much lower in the 29

“We now know that HIV treatment and primary prevention modalities will have to be used in an integrated manner within the field,” says Gina Brown, a senior staff member at the NIH Office of AIDS Research, at the close of the Sydney conference, according to a news summary by aidsmap, an HIV/AIDS information clearinghouse. “It is becoming clear that there needs to be an end-to-end plan that runs from discovery to delivery of each approach, and that this is unlikely to be achieved by the continued ‘silo-ization’ of the field.”

A program committee is being convened to plan the biennial conference. In the meantime, the 2012 AIDS Vaccine Conference in Boston will be held as planned as will the 2013 AIDS Vaccine Conference in Barcelona. Snow says the idea of a biennial conference that maximizes resources, brings together scientists with similar goals, and inspires cross-fertilization of research and development is welcome. “Recent breakthroughs in vaccines and other HIV prevention technologies have shown that we must consider implications and synergies as we continue to move HIV vaccine research forward,” says Snow.

He says the Enterprise plans to meet with a variety of stakeholders in the coming months to determine how best to achieve the benefits of an integrated meeting while keeping true to the Enterprise’s mission of advancing AIDS vaccine science. —*Regina McEnerly*

participants in both arms of the trial who acquired HIV. Only about a third of them had detectable levels of TDF when investigators first identified HIV antibodies in their blood plasma. “Even in visits prior to seroconversion, these people were not taking their drug as often as those who did not seroconvert,” Donnell said.

Yet adherence does not always predict PrEP efficacy. In her sub-analysis, Donnell noted that nine of the HIV-infected participants who had been assigned to either the TDF or Truvada arms did have detectable levels of TDF. Eight of them had drug levels that were consistently high or detectable throughout the follow-up—an indication the drug was being taken faithfully. “Of course, we don’t know what the level of the drug was at the time they got HIV infected,” said Donnell.

Baeten said that while adherence is arguably the primary determinant of PrEP efficacy, there are important hypotheses to consider about how biologic factors may diminish protection. He said pharmacokinetic studies have found that oral TDF achieves 10-fold or higher concentrations in rectal tissue compared to vaginal tissue, suggesting that PrEP may be more sensitive to non-adherence in women whose primary risk exposure is through vaginal sex, compared with men who have sex with men. He said sexually transmitted infections, genital inflammation and other factors that might increase the risk of HIV acquisition among high-risk heterosexual women could be interacting with PrEP to decrease its efficacy. “Research needs to assess whether this hypothesis can be supported,” he said. ■

Richard Jefferys is Coordinator, Michael Palm Basic Science, Vaccines & Prevention Project at the Treatment Action Group.

Research BRIEFS

Predicting the Antiviral Activity of Three-drug Combinations in HAART

Highly active antiretroviral therapy, or HAART, typically combines three antiretroviral drugs (ARVs) as a first-line treatment for HIV infection. It is a very effective way to keep HIV replication under control. So effective, in fact, that the best drug combinations suppress HIV to undetectable levels in most individuals.

What remains relatively unclear is why some but not other drug combinations work. Further, there is still no systematic method for determining which drugs to combine to get the most effective treatments.

Robert Siliciano, a professor of medicine at Johns Hopkins University School of Medicine, and his colleagues seem to have solved both problems. In a paper published in *Nature Medicine*, they describe a model that predicts how much HIV inhibition might be achieved by any three-drug combinations of the 19 most commonly prescribed ARVs (*Nat. Med.* 18, 446, 2012).

To create this model, they first measured in an *in vitro* assay how much each of the 19 ARVs inhibit a single round of HIV replication. They did this for several different concentrations of each drug and plotted out their results in a graph—generating a dose-response curve that describes how viral inhibition varies with each concentration of every studied drug. They found that the shape of the curves differed between the different ARVs. This highlights the importance of using dose-response curves in determining antiviral activity, Siliciano says, because these ARV-specific differences would likely have been missed by the way researchers have previously used to describe antiviral activity: by only focusing on one drug concentration, the so-called IC50, which results in 50% viral inhibition.

Next, the researchers determined the dose-response curves for almost all possible two-drug combinations of those 19 drugs, and compared them with the predictions of the relatively simple mathematical models researchers currently use to predict the potential efficacy of drug combinations. They found that, in more than 40% of the cases, these existing models of combined drug effects were unable to correctly predict their experimental results.

According to Siliciano, these existing models are too simplistic because they are based on two extreme scenarios. One model

assumes that the drugs to be combined target the same process in the HIV life cycle, in which case their pairing would have only an additive effect; the other assumes that two drugs target completely different processes in the HIV life cycle, in which case their combined effect should be multiplicative. Siliciano and his colleagues found, however, that in more than 40% of cases, the drug pairs they measured had effects that did not precisely fit either of these scenarios. “The existing models were so bad that we developed our own,” says Siliciano.

To do so, he and his colleagues used the results of their dose-response measurements to determine just where between the extremes the effects of various drug pairs really fell. They then used this information to calculate the inhibitory potential of three-drug combinations, by pulling together what they knew about the inhibitory effects of the drugs in any given three-drug combination alone and their pairwise combinations.

When they tested their predictions for 10 three-drug combinations in their *in vitro* assay, they found that this predicted HIV inhibition much better than the existing models. Their predictions also correlated very well with the outcomes of 47 clinical trials of three-drug combination regimens.

Before this study, there was no good way to predict the activity of three-drug combinations, says Ruy Ribeiro, a research scientist at the Los Alamos National Laboratory who models HIV treatment and wrote a commentary on the study in the same issue of *Nature Medicine*. “This gives some sign posts to what drug combinations to try in clinical trials,” he says. The new approach could also make it easier for researchers to identify less expensive drug regimens for use in developing countries, Ribeiro adds, allowing them to devise regimens that cost less but have potentially equivalent effects.

Siliciano, for his part, hopes to mainly use the new approach to improve management of individuals with drug resistance. This should now be possible, he says, by taking into account another recent finding by his group that suggests that resistance mutations in HIV can alter the shape of the dose-response curve (*Proc. Natl. Acad. Sci.* 108, 7613, 2011). —*Andreas von Bubnoff*

CD4⁺ T Cells Can Control Viral Load by Directly Killing HIV-infected Cells

CD4⁺ T cells are central players in the body’s immune response. Often called “helper” cells, they orchestrate the assault on invading pathogens: They stimulate the development of antibodies in B cells, and send signals to CD8⁺ T cells, which detect and destroy other infected cells, making them better killers and stimulating the forma-

tion of memory cells. But CD4⁺ T cells are also the primary targets of HIV, which is why they are not believed to play a leading role in controlling infection by that virus. Instead, CD8⁺ T cells are thought to be the primary agents of HIV suppression, deploying so-called “death” molecules such as perforin, which pops open infected cells,

and granzyme B, which forces them to commit suicide.

But helper T cells have recently also been found to play a somewhat more violent part in controlling infections by other viruses, such as influenza. Far from being solely coordinators and assistants, helper T cells participate directly in the killing of infected cells in such cases.

This made Ragon Institute researcher Hendrik Streeck and his colleagues curious about whether some CD4⁺ T cells might be similarly disposed in HIV-infected people. In a recent paper, they report that the answer, at least in some cases, appears to be yes (*Sci. Transl. Med.* 4, 123ra25, 2012).

Streeck and his colleagues looked at blood samples from 11 volunteers, starting at the time when they had just been infected with HIV. All of them had roughly the same viral load until about two months after they contracted the infection. But four months after that, six of the individuals suppressed their set-point viral load at a level 10-times lower than that of the other five.

Two months after infection—before one group developed a lower set-point viral load—the CD8⁺ T-cell responses were virtually identical in the two groups, but the researchers already found distinct differences in the HIV-specific CD4⁺ T-cell responses of the people who would later control their infections more effectively. These individuals had a much larger fraction of HIV-specific CD4⁺ T cells that carried granules containing granzyme A, a death molecule related to granzyme B, which CD8⁺ T cells use to kill infected cells. What's more, high granzyme A levels in HIV-specific CD4⁺ T cells correlated with about a one-year delay in the onset of disease progression.

This suggested that, like CD8⁺ T cells, CD4⁺ T cells can kill HIV-infected cells. In subsequent experiments, the researchers found that, indeed, HIV-specific CD4⁺ T cells taken from the people who better controlled the virus recognized and killed HIV-infected macrophages *in vitro*. They also found that HIV-specific CD4⁺ T cells could inhibit HIV replication in infected CD4⁺ T cells *in vitro*. This inhibition did not occur when the MHC class II receptor HLADR was blocked with an antibody, which is consistent with the fact that CD4⁺ T cells are activated by peptides presented by MHC class II receptors on the surface of antigen-presenting cells.

Together, this suggests a model where HIV-infected cells such as CD4⁺ T cells or macrophages present HIV peptides via two pathways: through MHC class I molecules to activate CD8⁺ T cells, and through MHC class II molecules to activate CD4⁺ T cells (see figure, right). This means, Streeck says, that even if the virus inhibits one pathway—for example by downregulating MHC class I molecules or by developing escape mutations—effective recognition and lysis of the infected cell could still be achieved through the other pathway that involves MHC class II and CD4⁺ T cells.

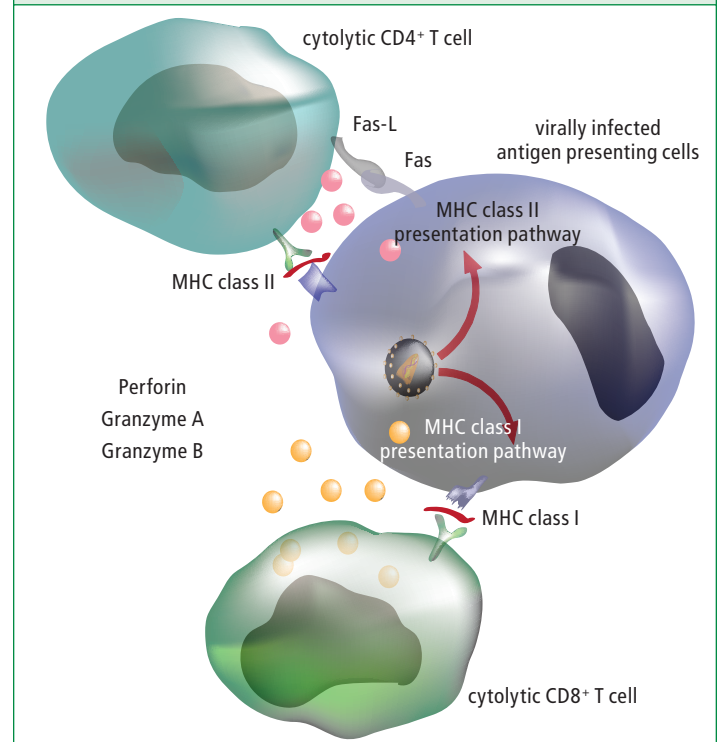
Streeck says these results are consistent with a recently published study that suggests that in the RV144 HIV vaccine trial—the only one so far that has provided evidence of efficacy—the modest protection observed in vaccinated volunteers was in part associated with HIV-specific CD4⁺ T cells that expressed certain molecular markers that indicate that they were able to kill infected cells (*J. Immunol.* 188, 5166, 2012). Together with his recent study, Streeck says, this suggests that the induction of a CD4⁺

T-cell response by a vaccine might translate into viral load control and also into protection from infection.

Nichole Klatt, a research fellow in Jason Brechley's laboratory at the National Institute of Allergy and Infectious Diseases, who co-wrote a commentary on the study in the same issue of *Science Translational Medicine*, calls the findings “very important.” Measuring HIV-specific cytolytic CD4⁺ T cells during acute infection, she suggests, could help determine disease progression. She adds that such cells may also be a valuable target for vaccination strategies. Klatt notes, however, that because CD4⁺ T cells are also HIV's primary target, any vaccine that induces their proliferation could also serve up more host cells for the virus. “Further studies should be performed in the SIV model,” says Klatt, to assess the safety of expanding HIV-specific CD4⁺ T cells to ensure that there are no “increased infection rates due to increased targets.” —*Andreas von Bubnoff*

Model for dual-pathway killing by CD4⁺ and CD8⁺ T cells

HIV-1 can establish infection within cells that express both MHC class I and class II molecules, such as antigen-presenting macrophages. Antigen presentation by these molecules is critical for recognition by circulating cytolytic CD4⁺ and CD8⁺ T cells. Even if the virus were to inhibit recognition through one pathway—for instance by downregulating MHC class I molecules or via the acquisition of escape mutations—effective recognition and lysis of the infected cell (via secretion of molecules like perforin or granzyme as indicated by the pink and yellow spheres) could still be achieved through the other pathway. Adapted from *Expert Review of Vaccines*, December 2010, Vol. 9, No. 12, Pages 1453-1463 with permission of Expert Reviews Ltd.



The Next Step In Our Evolution

Since 1996, *IAVI Report* has been the source of information on AIDS vaccine research and development.

VAX has been keeping a general audience informed on AIDS vaccine research since 2003.

Today, the redesigned, interactive iavireport.org puts all the news and science at your fingertips, featuring:

- VACCINE TRIALS DATABASE
- ORIGINAL VIDEOS
- CONFERENCE REPORTS
- LATEST NEWS AND FEATURES
- COMPLETE *IAVI REPORT* AND *VAX* ARCHIVES

Log on today.

www.iavireport.org

