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**Reports from  
CROI and Keystone**

## EDITOR'S LETTER

It isn't often that a toddler dominates discussion at a scientific conference—unless, of course, the conversation takes place between a pair of doting grandparents who also happen to be researchers.

But with the report that a 30-month-old Mississippian appears to have been “functionally” cured of HIV by early and aggressive treatment, that's exactly what happened this year at the 20<sup>th</sup> Conference on Retroviruses and Opportunistic Infections (CROI). The finding fueled much water-cooler conversation at the gathering and inspired more than a few breathless news stories. Those of you who missed the conference will be happy to know that *IAVI Report* had not just one but *two* correspondents there to report on the matter.

In this issue, our contributor Richard Jefferys describes the remarkable case and other notable HIV cure-related research presented at the conference. Meanwhile, Regina McEnery, who blogged about the toddler news as it broke, reports on CROI talks and presentations that touched on HIV prevention. Andreas von Bubnoff, for his part, shares a story from the Keystone symposium on HIV vaccines that focuses on, well, HIV vaccine research. If things like broadly neutralizing antibodies, passive immunization and virus-like particles set your pulse racing, we invite you to immerse yourself in his report.

Our shorts this year include items on the WHO's HIV guidelines, a research report on HIV Tat as a possible target for protective neutralizing antibodies, and the launch of a new replicating viral vector HIV vaccine candidate in clinical trials. We also share news of a protest, planned (as this magazine went to press) for April 8<sup>th</sup> in Washington, D.C., against cuts to federal funding for health research. But that's just a heads-up: Ms. McEnery will be there to report on what—if anything—happens for the *IAVI Report* blog. Read all about it on our website—and, please, keep coming back. You'll notice how lively our blog has become, with frequent updates on scientific research, policy and many other matters of relevance to HIV prevention.

I sincerely hope you enjoy our first issue of 2013.

– UNMESH KHER



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The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996, 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](http://www.iavi.org).

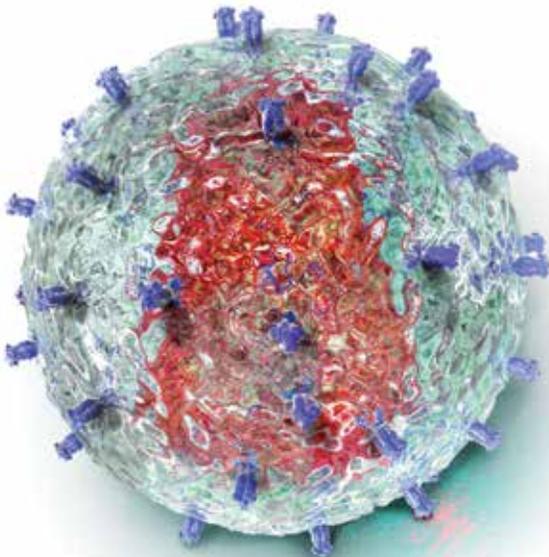
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# IAVIReport

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## [ ON THE COVER ]

Mirrored image of a cross-section of one half of a lymph node that was isolated from a transgenic mouse that produces a fluorescent protein in cells that express CD11c (green), a cell surface protein that's predominantly expressed by dendritic cells (DCs). Researchers also stained the lymph node with fluorescently labeled antibodies to CD8, a marker expressed by CD8<sup>+</sup> T cells and some types of DCs (blue); to CD11b, a surface protein expressed by some DCs and by macrophages, neutrophils and monocytes (yellow); and to reticular fibers, which separate the lymph node into different zones (red). Cells that express combinations of these markers appear in mixed colors. For example, while DCs that only express CD11c are green, some DCs that also express CD8 (blue) appear in cyan. In a recent study, NIAID researchers used computer-based image analysis of these color combinations to map different cell types in the lymph node that can't be distinguished by the naked eye (*Immunity* **37**, 364, 2012). This showed that lymph nodes are more organized into different functional areas than previously appreciated.

Image courtesy of Michael Y. Germer and Ronald N. Germain, NIAID. A related version of this image appeared in *Immunity* **37**, 364, 2012.

# A Toddler *STOLE THE SHOW*

The report of a child functionally cured of HIV stirred up the typically staid proceedings of the Conference on Retroviruses and Opportunistic Infections

By Richard Jefferys and Regina McEnery

The diminutive person who loomed largest at CROI was not in attendance.

An anonymous child from Mississippi, just 30 months old, drew headlines and became grist for multiple corridor confabs at the 20<sup>th</sup> Conference on Retroviruses and Opportunistic Infections (CROI) in Atlanta when it was reported—at a press conference before the science meeting opened, and then more fully during a session on HIV eradication—that the child had been functionally cured of HIV infection by early antiretroviral therapy (ART). The case was described by Deborah Persaud, a researcher and clinician-scientist at the Johns Hopkins Children’s Center who has decades of experience in pediatric HIV.

The story begins with an unnamed pregnant woman who received no prenatal care, and whose HIV infection was not diagnosed until she was in labor, leaving no time to administer AZT to reduce the risk of mother-to-child transmission. The rural hospital where the baby was delivered referred the infant to Hannah Gay, a clinician at the University of Mississippi Medical Center. Due to the high risk that HIV had been transmitted to the child, Gay decided to initiate a three-drug treatment regimen of AZT, 3TC and nevirapine, rather than the more standard dual combination of AZT and nevirapine that is typically given to children exposed to HIV.

That turned out to be a good call: a sample taken at 30 hours after birth tested positive for HIV DNA, while a second taken at 31 hours documented a viral load level of 19,812 copies. Therapy was then altered slightly after seven days, when the protease inhibitor lopinavir was substituted for nevirapine.

Over the course of the next several weeks, repeat viral-load testing revealed stepwise declines

to 2,617 copies, 516 copies, 265 copies and then less than 48 copies, which is below the limit of detection of the assay they were using. About 18 months later, however, both mother and child were lost to care. When they returned, it transpired that therapy had been stopped. (Due to privacy concerns, Persaud declined to explain the exact circumstances.)

When Gay performed a new round of viral load measurements, expecting the typical rebound that has been described in many studies of pediatric ART interruption, she was surprised to find that HIV remained undetectable. Gay contacted Katherine Luzuriaga, professor in the division of Pediatric Immunology & Infectious Diseases at the University of Massachusetts Medical School, and an expert in the immunology of pediatric HIV infection. Luzuriaga in turn contacted Deborah Persaud, due to her experience in measuring latent HIV reservoirs in children. Persaud was the first to describe latent HIV in children (*J. Clin. Invest.* 105, 995, 2000).

Genetic studies confirmed the mother-child relationship and demonstrated that neither possessed known beneficial human leukocyte antigen alleles or protective CCR5 genotypes that could have made the child less susceptible or resistant to HIV.

Meanwhile, multiple laboratories searched for trace amounts of HIV in samples taken at 24 or 26 months of follow-up: Doug Richman at the University of California at San Diego employed digital droplet PCR to quantify HIV DNA in peripheral blood mononuclear cells. Tae-Wook Chun at the US National Institute of Allergy and Infectious Diseases (NIAID) looked for HIV RNA in plasma using a modified version of the Roche AMPLICOR test, which carries a limit of detection of two copies per ml of blood. Michael Piatak at the Fred-

erick National Laboratory for Cancer Research—a branch of the National Cancer Institute—also assessed viral RNA in plasma using a single-copy assay, and Persaud herself looked for replication-competent HIV in resting CD4<sup>+</sup> T cells using a co-culture approach.

Although the majority of these tests were negative, in two instances HIV proviral DNA was detected at levels slightly above the lower limit of the assays, suggesting that residual virus might have remained in the child's blood or tissue. The single-copy assay for HIV RNA—so named for its detection limit of 0.3 copies per ml of plasma—also scored positive for one HIV RNA copy/ml at the 24-month time point. Importantly, Persaud was unable to detect any replication-competent HIV in a sample of 22 million resting CD4<sup>+</sup> T cells.

To complement the virology, Luzuriaga undertook an array of immunological investigations. CD4<sup>+</sup> T-cell counts and immune activation levels were comparable to age-matched HIV-uninfected children. No HIV-specific antibody responses were detectable by ELISA and Western Blot tests, and an analysis of CD4<sup>+</sup> T-cell or CD8<sup>+</sup> T-cell responses to Gag and Nef peptide pools also came up empty.

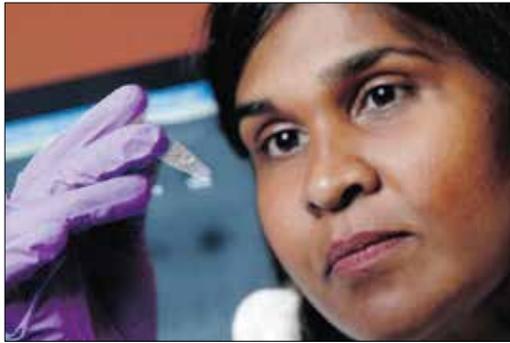
Persaud and her colleagues concluded that the case represents the “first well-documented case of ‘functional cure’ in an HIV-infected child.”

But, inevitably, after the data came questions and debate. David Margolis, director of the School of Medicine at the University of North Carolina and an HIV cure researcher, pointed out that the term functional cure was originally suggested to mean containment of HIV by virus-specific immune responses. Such responses have not yet been detected in the child.

“I would just like to point out that I don't really like the term functional cure because I think it is very confusing for the public and for patients,” Margolis told Persaud during the question-and-answer period following her presentation.

Persaud noted that appropriate terminology for the pediatric population is not yet clear and, because follow-up off ART was relatively short—a little over 10 months—the researchers chose this term to try and convey the uncertainty regarding whether a complete cure had been achieved.

Another questioner asked whether the transfer of maternal cells containing HIV into the baby could explain the viral load readings. But Persaud had considered the possibility and calculated that an implausibly large transfer of around 250 ml of maternal blood across the placenta would be required to explain the amount of HIV RNA detected.



Deborah Persaud

Additional questions posed at the CROI press conference included whether HIV might have been cleared even without ART, as was reported in a few rare published studies from the 1990s (*N. Engl. J. Med.* 332, 833, 1995; *Lancet* 347, 213, 1996). Persaud cited an analysis of viral genetic sequences from 1998, which concluded that sample contamination and mix-ups appeared to explain the majority of these findings, and that conclusive evidence for transient infection was lacking (*Science* 280, 1073, 1998).

A suggestion commonly heard in CROI hallway discussions was later articulated in a *Wall Street Journal* op-ed piece by Mark J. Seidner, a postdoctoral fellow in the division of infectious diseases at Massachusetts General Hospital and Harvard Medical School. Seidner, who works in Uganda, suggests ART did not cure HIV infection in the infant but acted as a form of post-exposure prophylaxis (PEP).

Yet, as Persaud explained at CROI, the detection of viral RNA and DNA within 48 hours after birth is widely accepted as evidence that infection occurred *in utero*. Further, studies involving viral load measurements in large numbers of infants receiving PEP have not documented any similar examples of progressive declines followed by an absence of evidence of infection (*J. Pediatr.* 160, 60, 2012).

To some extent, this represents a debate over exactly what constitutes established HIV infection. Persaud and colleagues hypothesize that early treatment prevented the formation of a latent HIV reservoir in the infant; for some scientists, this amounts to a kind of prophylaxis more than a cure.

In fact, researchers are probing this very question in HIV-infected adults. The largest study thus far assessing the impact of earlier treatment on the size of reservoirs is being conducted in Thailand by Jintanat Ananworanich (see *Is It Ever Too Early?*, *IAVI Report*, Sep.-Oct. 2012).

## [ SEARCHING FOR THE SEQUEL ]

A late-breaker poster presentation from the newly formed European HIV Cure and Transplant Consortium (EHCTC) provided an update on attempts to repeat the cure of HIV infection obtained in Timothy Brown, widely known as the “Berlin patient” for undergoing two bone marrow transplants there to treat his leukemia. Results appear to be mixed.

Two HIV-infected individuals on antiretroviral therapy (ART) with different types of cancer were identified as candidates to receive cord blood-derived stem cells from donors homozygous for the CCR5 $\Delta$ 32 mutation. In one of them—a man from the Netherlands who was kept on ART throughout—the transplant was followed by an apparent rapid decay in HIV DNA levels. But he died two months later due to recurrence of the cancer and severe pneumonia.

In the second case, a man from Madrid, cord blood stem cells that were wild type for CCR5 had to be used due to concerns about the viability of cells from the CCR5 $\Delta$ 32 donor. In this case, a much slower decay of HIV RNA and DNA was seen, and the individual remains on ART but, happily, is in remission from the cancer. The EHCTC continues to work to identify additional candidates for CCR5-negative stem cell transplants. —RJ

They have found that the earlier treatment is started, the smaller the reservoir size in blood and colon six or 12 months later.

In terms of the broader implications of the case, Persaud described plans to conduct studies of prompt HIV treatment in infected infants through the International Maternal Pediatric Adolescent AIDS Clinical Trials group, in order to assess whether the results can be duplicated. In a separate poster presentation at the conference, Luzuriaga presented data on five HIV-infected youths (average age 16) who have been on ART since around two months of age and show no detectable levels of replication-competent virus. Luzuriaga suggested that such individuals are prime candidates for careful studies of treatment interruptions and interventions that seek to achieve a cure.

Amidst the debate and controversy, there was one very clear take-home message from the work of Persaud and colleagues: the pediatric population should be considered an essential part of the cure research agenda.

## No cure-all treatment

CROI 2013 also saw the public debut of data from the first multi-dose trial of a potential latency-reversing agent, the anticancer drug vorinostat (SAHA). Sharon Lewin, Professor and Director of Infectious Diseases at Alfred Hospital and Monash University in Melbourne, previewed the toxicity data at last year’s meeting (see *A Slew of Science In Seattle, IAVI Report*, Mar.-Apr. 2012). This time, Lewin described the effects of 14 days of vorinostat on HIV RNA expression in the latent reservoirs of 20 participants on long-term suppressive ART.

Echoing results from the single-dose study by Margolis published last year (*Nature* 487, 482, 2012), Lewin’s regimen induced a statistically significant 2.78-fold increase in cell-associated HIV RNA. However, Lewin found that there was no reduction of HIV reservoirs as measured by proviral DNA levels, consistent with studies from the laboratory of Robert Siliciano at Johns Hopkins University. Those studies found that induction of HIV RNA expression alone may be insufficient to cause the death of latently infected cells (*Immunity* 36, 491, 2012).

Siliciano himself delivered the opening plenary talk on some of the key challenges in cure research. In addition to the potential need for interventions, such as therapeutic vaccines to deliver the coup de grâce to latently-infected cells induced to express HIV RNA, Siliciano highlighted several other issues that continue to trouble researchers.

Among them is the question of whether laboratory cell line models of HIV latency accurately reflect what occurs *in vivo*. Siliciano noted that latency-reversing agents like vorinostat can show differential activity in different experimental models. To try and definitively assess the activity of candidate anti-latency approaches, Siliciano is now collaborating with Margolis to collect large numbers of latently-infected resting CD4<sup>+</sup> T cells from HIV-positive individuals via leukopheresis. Until now, the relative scarcity of these cells has precluded this type of analysis.

The accurate measurement of HIV reservoirs in HIV-infected individuals on ART is another difficult task. While results from various assays are commonly reported, exactly how they relate to each other has not been clear. Siliciano cited a recent comprehensive analysis that involved multiple laboratories testing samples from well-defined cohorts of people with suppressed viral loads. The study found no correlation between the gold standard viral outgrowth test for replication-competent HIV and most assays for viral DNA or RNA (*PLoS. Pathog.* 9, e1003174, 2013). Because viral outgrowth is time-consuming and cumbersome to measure, Siliciano’s laboratory is now working on a simplified version.

Siliciano also addressed a related question: What amount of reduction in the HIV reservoir might prevent viral load rebound after ART interruption, and for how long? His colleagues, led by Alison Hill from Harvard University, have developed a mathematical model that suggests at least a three log decline in the reservoir of latently infected cells would be required to prevent rebound for a period of several years, but late recrudescence of viremia might still be possible even with greater reductions.

## Genetic protection

One proposed method of limiting HIV’s ability to replicate in the absence of ART is gene therapy. The idea is to genetically modify vulnerable cells in ways that prevent the virus from gaining entry or, short of that goal, replicating. The widely publicized case of Timothy Brown, the lone adult cured of HIV infection, has given impetus to this strategy: Brown received a transplantation of cells from a donor homozygous for the CCR5 $\Delta$ 32 mutation, abrogating expression of this key HIV co-receptor.

For Brown, however, stem cell transplantation was medically required to treat his cancer, and the cells he received came naturally modified. Genetically modifying all immune cells in otherwise healthy HIV-infected individuals, on the

other hand, presents daunting challenges. On the plus side, Patrick Younan, a post-doctoral research fellow in the laboratory of Hans-Peter Kiem at Fred Hutchinson Cancer Research Center, described intriguing results from a small pig-tailed macaque study that hints that modification of all CD4<sup>+</sup> T cells may not be necessary.

The experiment involved transplantation of stem cells modified with a gene encoding a peptide inhibitor of virus entry named C46—which works very much like the viral entry inhibitor drug Fuzeon—in tandem with a gene for green fluorescent protein (GFP) to allow identification of successfully altered cells. Two macaques received transplants with both C46 and GFP, while two controls were given cells containing only the GFP marker. All animals were subsequently challenged with the SIV/HIV hybrid SHIV 89.6P. Younan showed that, as is typical in studies using this virus, CD4<sup>+</sup> T-cell counts plummeted during acute infection.

However, T-cell counts rebounded in the two recipients that received C46-modified cells. The viral loads in these animals dropped to levels significantly lower than controls—320-fold lower in one case and 1,477-fold lower in the other.

Analyses of the proportion of gene-modified CD4<sup>+</sup> T cells to the total T-cell count prior to challenge demonstrated blood levels of 20% and 55%, respectively. During the acute phase of infection, levels rose to 92%, consistent with positive selection of virus-resistant CD4<sup>+</sup> T cells. However, the percentage subsequently declined to pre-challenge levels in parallel with an increase in numbers of unmodified CD4<sup>+</sup> T cells.

Younan and colleagues hypothesized that gene modification might be protecting SHIV-specific CD4<sup>+</sup> T-cell responses and allowing them to mediate superior helper functions, leading to better immune control of viral replication. The result is improved protection of unmodified, susceptible cells. Supporting their suspicions, SHIV-specific CD4<sup>+</sup> T cells were undetectable in controls, but responses to Env, Gag, Nef and Pol were present in the recipients of gene-modified cells. Further investigation revealed that approximately 85% of these SHIV-specific CD4<sup>+</sup> T cells were descendants of modified cells. This implies that gene therapies may not need to protect the entire pool of susceptible cells in order to be effective. Additional experiments are now underway in hopes of confirming and extending the findings.

Novel evidence of the benefits of protecting virus-specific CD4<sup>+</sup> T cells was presented by Adrienne Swanstrom, a graduate student at the

University of Pennsylvania. Swanstrom outlined an unusual strategy to gain insight into the role of infection of CD4<sup>+</sup> T cells in SIV (and by extension, HIV) pathogenesis.

Swanstrom and colleagues created an altered version of the highly pathogenic SIVmac239 that does not bind the CD4 molecule, while retaining the ability to interact with the co-receptor CCR5. This virus, designated iMac-ΔD385, was used to challenge two macaques. Peak viral loads were comparable to those typically observed with wild-type SIVmac239. But they were subsequently rapidly reduced and maintained below the limit of detection, between 30 and 50 copies/ml. No loss of CD4<sup>+</sup> T cells occurred in blood, gut, or lymph nodes.

In assessing where infection was occurring in gut and lymph node samples, SIV p27 staining identified three populations: CD3<sup>+</sup> T cells, CD68<sup>+</sup> macrophages, and an as-yet unidentified CD3<sup>-</sup>, CD68<sup>-</sup> cell type. Localization of infection in lymph nodes showed a reverse pattern to that seen in pathogenic SIV, with few infected cells in the cortex and the bulk of infection occurring in the medulla.

Analysis of humoral immune responses revealed that the decline in viral load coincided with the generation of neutralizing antibodies. These antibodies, *in vitro* studies showed, had broad activity against iMac-ΔD385, SIVmac251 and the heterologous SIVsmE660. Such antibody responses are rarely seen in wild-type SIVmac239 infection.

The results appear consistent with the hypothesis of Swanstrom and colleagues that sparing CD4<sup>+</sup> T cells from infection would improve adaptive immunity. Investigations in additional macaques are now planned in order to evaluate humoral and cellular immune responses in more detail, and study whether animals infected with iMac-ΔD385 can resist challenge with pathogenic SIV.

## Scars of inflammation

The laboratory of Ashley Haase at the University of Minnesota has pioneered research into the association between HIV infection and fibrotic (scarring) damage to the lymph tissue (*Semin. Immunol.* 20, 181, 2008). At CROI, Joyce Sanchez, a research fellow in the laboratory, presented results from studies measuring lymph tissue fibrosis in HIV controllers.

Sanchez explained that fibrosis occurs in both lymph nodes and gut-associated lymphoid tissue (GALT) in HIV infection due to persistent immune activation and inflammation. The extent of the damage increases as disease progresses. Imaging analyses of the total area of lymph tissue samples

occupied by collagen permits the quantification of such damage. Contrary to expectations, Sanchez found that HIV controllers displayed significantly increased levels of lymph tissue fibrosis compared to uninfected controls, regardless of whether viral load was undetectable or low (less than 2,000 copies/ml). There were also no statistically significant differences in fibrosis between the HIV controllers and HIV-infected groups with progressive disease either on or off ART.

Sanchez concluded that even the relatively low levels of viremia and immune activation seen in HIV controllers are sufficient to sustain

fibrotic damage to lymphoid tissue. On a less pessimistic note, Sanchez highlighted recent results obtained from Timothy Brown, whose GALT displayed fibrosis that matched HIV-uninfected controls: the percentage of tissue occupied by collagen was 6.8%, compared to 7% in the uninfected group and 15.9% in HIV controllers. The implication, Sanchez noted, is that curing HIV infection can reverse the scars of the past.

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

## ...But HIV prevention got some play at the conference

Perhaps because the annual Keystone symposia on HIV Vaccines was held a month early this year—in February—CROI offered far fewer oral abstracts and posters related to vaccine science than in previous years. Even the plenaries, which set the tone for the conference and highlight big-picture science themes, ignored AIDS vaccines.

Still, CROI did offer up some jewels from the vaccine front, including fresh data from efforts to develop an AIDS vaccine based on a persistent replicating rhesus cytomegalovirus (rhCMV) viral vector containing several simian immunodeficiency virus (SIV) genes. Louis Picker, a professor of pathology at Oregon Health & Science University, who has been studying rhCMV vectors for nearly a decade, delivered fresh data in an expansive 30-minute talk that bolstered what his previous experiments suggest: some Indian rhesus macaques vaccinated with the rhCMV vectors, either alone or in combination with another viral vector vaccine, not only suppressed but cleared SIV following intravaginal challenge.

Picker conducted what he called an “acid test” for induced immunity—injecting naïve rhesus macaques with 60 million peripheral blood mononuclear cells taken from five SIV-infected animals who had been able to suppress the virus for at least 17 months post-challenge. When the cells from SIV-infected monkeys on fully suppressive highly active antiretroviral therapy or from monkeys able to control SIV without antiviral drugs were injected into SIV-uninfected animals, their transfer led to rapidly detectable infection. But in the case of the rhCMV vaccine-protected animals, no infection was detected.

“The implication is that there is no residual SIV in the rhCMV/SIV vector-vaccinated, long-

term protected animals,” said Picker. “The SIV infection, which was demonstrable there early on, after challenge, is now gone, cleared, nada.”

These results build on previous work by Picker and colleagues that compared the immunogenicity of rhCMV vaccine candidates in a four-arm trial involving 61 macaques previously exposed to CMV. Twelve macaques were given the rhCMV/SIV viral vector-based vaccine, an equal number received an rhCMV/SIV vector-based candidate followed by a replication-defective adenovirus serotype 5 (Ad5) vector-based candidate encoding the full SIVmac239 genome, and nine received a DNA prime/Ad5 boost (encoding the full SIVmac239 genome) regimen. The 28 control animals received no vaccine. That study found that 12 of 24 macaques vaccinated with the replication-competent rhCMV viral vaccine candidate encoding the SIV proteins Env, Pol, Gag and Vpr/Vpx demonstrated early and complete control of viral replication for more than a year after repeat, homologous, low-dose SIVmac239 challenge (*Nature* 473, 523, 2011).

The study found no demonstrable differences in the number of challenges needed to infect the animals. And the remaining 12 macaques that received the CMV vaccine only and five of the 12 macaques that received the rhCMV/Ad5 vaccine combo were not able to control viral replication.

Exactly why some of the rhCMV-vaccinated macaques hit the biological equivalent of a grand slam, while others struck out, is not entirely clear. In previous studies, Picker and colleagues found that the persistent replicating vector induces effector memory T cells, which Picker suggests are better at protecting from virus in mucosal tis-

sues than the central memory T cells induced by non-replicating vectors (see *Nat. Med.* 15, 293, 2009). Picker and colleagues also determined the mechanism that enabled CMV—an almost ubiquitous virus—to overcome pre-existing immune responses and superinfect monkeys already infected with the virus. They found that to superinfect its host, rhesus CMV needs genes that prevent major histocompatibility complex (MHC) class I of infected host cells from presenting CMV proteins to CD8<sup>+</sup> T cells (*Science* 328, 102, 2010).

Picker thinks one of the reasons half of the rhCMV-vaccinated monkeys in his studies were unable to control SIV infection could be because they failed to generate enough effector memory T cells early enough. He said necropsies conducted in the protected animals showed responses “higher than what we see even in general vaccinees.” Picker’s lab is now working with the Nonhuman Primate Consortium funded by the US National Institute of Allergy and Infectious Diseases (NIAID) to look at the animals eight days after onset of infection. This is when you see responses in monkeys “going one way or another,” said Picker.

Picker said genetic polymorphisms in monkeys might also explain why roughly half the monkeys across five different trials have been able to suppress SIV while the other half have not. “But in some ways, it doesn’t really matter because in humans it might be 0% or 100%,” said Picker. “We’re not going to know that until we take [the CMV vector] into clinical trials.”

In any case, Picker said the CD8<sup>+</sup> T-cell responses in the rhCMV-vaccinated animals appeared to violate all the rules. He noted that the responses are extraordinarily broad, targeting many different SIV epitopes found in all or most vaccinated animals—42 animals tested by Picker had a 100% response to the same epitope. The CD8<sup>+</sup> T-cell responses also seem to rely on MHC class II molecules to recognize the virus—a trait more associated with CD4<sup>+</sup> T cells—though Picker said the CD8 responses in his model are also capable of mediating SIV-infected cell recognition via class I molecules.

“Basically, with these vectors we can actually program T-cell responses that have effector memory differentiation that have entirely non-overlapping T-cell epitope recognition,” said Picker. These findings, he said, might provide proof of principle that the cellular immune system can mediate potent clinically relevant protection, including viral clearance, “by itself, without antibodies, against a highly pathogenic lentivirus.”

## The Envelope, please

On the antibody side, University of Iowa researcher Hillel Haim presented findings from a recently published study that looked more closely at how the cleavage of the HIV Envelope gp160 protein affects its recognition by a wide range of antibodies, including broadly neutralizing antibodies (bNAbs) (*J. Virol.* 87, 1884, 2013).

Understanding this process will presumably enable researchers to design immunogens that more closely mimic the trimeric functional form of Env that appears on the viral surface, one that bNAbs must recognize to thwart infection.

Haim, who recently opened his own lab on the Iowa City campus, developed the premise for his research project while working as a post-doc in the lab of Joseph Sodroski, a professor of microbiology and immunology at the Dana-Farber Cancer Institute in Boston. Much of Sodroski’s work has focused on elucidating the structure and function of HIV Env in its various conformational states. Last year, he described an 11-Ångstrom structure of the unliganded HIV Env trimer using single-particle cryo-electron microscopy (*Nat. Struct. Mol. Biol.* 19, 893, 2012).

Because the Env spikes that adorn the surface of HIV are highly unstable and structurally dynamic, scientists, including Sodroski, have not been able to obtain a higher-resolution crystal structure of the unliganded Env trimer with methods more commonly used in structural biology. Knowledge of the atomic-scale structure of the Env spike could improve HIV drug design and remove much of the guesswork from selecting the best immunogens for vaccine candidates.

In the absence of a crystallized image of the unliganded trimer, Haim decided to examine the pattern of antibody binding to the Env trimer expressed on human osteosarcoma (HOS) cells, which allow clear differentiation between cleaved and uncleaved forms of Env. Haim and his colleagues used a cell-based ELISA system to measure binding of both cleavage-competent (wild-type) and uncleaved (mutant) Env against a panel of 16 bNAbs of varying breadth and potency. In a parallel test, they stabilized the HOS cells with the fixing compound glutaraldehyde to measure the effect of chemical fixation on the antigenicity of Env. “Fixation provides a snapshot of the different conformations that Env can assume at different times,” said Haim.

Haim said he and his colleagues found that both cleavage and chemical fixation were associated with an increase in binding by antibodies,

such as VRC01, that target exposed epitopes. But they found cleavage and chemical fixation to be associated with a decrease in binding by antibodies like b13 and 17b that target the less visible (cryptic) epitopes overlapping the CD4-binding and co-receptor-binding sites, respectively. This would suggest, said Haim, that cleavage results in structural changes to Env that decrease flexibility and affect antibody recognition.

Based on his experimental results, Haim has developed a mathematical model to predict how well antibodies neutralize HIV. The model incorporates the efficiency of antibody binding, the level of Env reactivity (a measure of the sensitivity of the Env to the bound antibody) and the degree of structural change (perturbation) that occurs when an antibody binds to Env—which he calls the perturbation factor (PF).

The degree of perturbation caused by each antibody is strongly associated with how exposed the target epitope is on Env. Antibodies with a low PF, like VRC01, have target epitopes that are always visible on Env (i.e., more frequently sampled), while antibodies with a high PF, such as 4E10, have target epitopes that are masked and harder to find because they are less frequently sampled.

Haim's model found that antibodies with a low PF neutralized both tier 1 and tier 2 viruses, compared to those with a high PF, which neutralized only tier 1 viruses. Haim said the model suggests that antibody recognition and the frequency at which the epitope targets of antibodies are sampled before binding both determine how potent an antibody will be.

In another talk, University of San Diego scientist Gabriel Wagner reported that the lack of broad and potent neutralizing responses to heterologous viruses might predispose HIV-infected individuals to superinfection. Wagner and colleagues measured the breadth and potency of neutralizing antibody responses before and after super-infection in a cohort of men who have sex with men (MSM) who were not receiving antiretroviral therapy. They then compared the responses to a matched cohort of individuals who had not been superinfected. The researchers used gene sequencing tools to estimate the frequency of superinfection in the MSM cohort.

They found that neutralizing antibody breadth and potency to a heterologous virus panel was significantly weaker three months after initial infection among those who would become superinfected. A year after infection, those who had become superinfected still had less neutralizing

breadth and potency to the heterologous viruses.

Wagner said the study provides evidence that a broad and potent neutralizing antibody response to heterologous virus may be a correlate of protection against superinfection and “likely critical in the preventive vaccine effort,” though he also said increased viral genetic diversity through superinfection does not seem to accelerate the response.

Cynthia Derdeyn of the Emory Vaccine Center at Emory University also presented recently published findings that shed light on how the family of PGT128-like antibodies develop (see *IAVI Report* March 7 blog, *Development of broadly neutralizing antibodies against HIV: It's complicated*). Previous studies suggest that the PGT128-like bNAbs isolated in HIV-infected individuals target a sugar residue connected with an amino acid at position 332, at the base of the so-called V3 loop of HIV's Envelope protein.

But Derdeyn's findings suggest the mere presence of a sugar residue at position 332 of HIV Env may not always suffice for the development of PGT128-like antibodies. Derdeyn said the same set of mutations can result in different outcomes, depending on when they appear. Not only are specific changes in Env likely to be important, she said, but neutralization breadth might also be programmed by sequential or simultaneous exposure to viral escape pathways, which result in a wave of antibody evolution.

## PrEP update

Two years ago, there was something of a PrEP rally at the CROI meeting in Boston, with upbeat talk about the biomedical prevention strategy (the acronym derives from pre-exposure prophylaxis) dominating scientific discussion and hallway conversation (see *PrEP Rally*, *IAVI Report*, Mar.-Apr. 2011).

This year, the news was a bit more sobering. Researchers wrestled with the latest spate of disappointing findings—this time from the Vaginal and Oral Interventions to Control the Epidemic (VOICE) trial of 5,029 high-risk women. The study was launched in 2009 from 15 sites in Uganda, South Africa, and Zimbabwe to evaluate the efficacy of daily topical and oral antiviral drugs in preventing HIV transmission among high-risk heterosexual women. The three products tested were tenofovir gel, oral tenofovir (TDF) and oral Truvada—a combination of tenofovir and emtricitabine.

Jeanne Marrazzo, a researcher from the University of Washington, which led the VOICE

trial, said analysis of blood samples from 773 women—including 185 who acquired HIV—suggests adherence was low across all study groups. Less than a third of the blood samples from women assigned to either the Truvada or oral TDF arms, and less than a quarter of samples from women assigned to the gel arm had detectable levels of drug. As might be expected, none of the daily topical or oral regimens evaluated in the trial could be linked to a decline in HIV acquisition.

Less expected and more alarming was what the trial revealed about HIV incidence in participating cohorts: it came to 5.7% across all groups, and climbed to as much as 8.8% among unmarried women from South Africa who are under the age of 25, which is a remarkably high rate of infection. “The rate of acquisition did not differ product to product,” noted Marrazzo. “Adherence was low across the bar.”

Marrazzo said behavioral analyses will try to get at the root of the poor adherence. “Obviously, we were disappointed,” she said. Trial investigators also plan to examine drug levels in vaginal fluid to see if there is any relationship between the use of the products—particularly the tenofovir gel—and efficacy.

Yet Marrazzo’s findings were not exactly unanticipated. In the fall of 2011, one arm of the trial was stopped for futility after a data safety monitoring board (DSMB) determined that it would be impossible to show any difference between oral TDF and placebo in preventing HIV infection (see VAX Sep. 2011 *Global News*). Two months later, the DSMB reached the same conclusion on the topical administration of 1% tenofovir gel, a microbicide. The remaining arm of the trial tested daily administration of Truvada.

NIAID, the primary funder of the trial—which was earlier projected by the Microbicide Trials Network to cost US\$100 million—noted that the DSMB found no safety concerns with oral TDF, which is currently used to treat HIV. The VOICE trial was sponsored by NIAID, the Microbicide Trials Network, Gilead Sciences (the manufacturer of tenofovir and Truvada), and CONRAD, a Virginia-based research institute developing contraceptive products and options to prevent HIV and other sexually transmitted infections.

The poor adherence mirrored findings from another trial, FEM-PrEP, which was also stopped early for futility (see *Vaccine Briefs, IAVI Report*, Mar.-Apr. 2011). The trial was conducted in

Africa and led by Family Health International (FHI) 360 in North Carolina. Tim Mastro, who oversees research and programs in global health, population, and nutrition at FHI 360, remarked at CROI that the PrEP findings are “sobering” and reflect a “tremendous disconnect between perception and willingness to take product.”

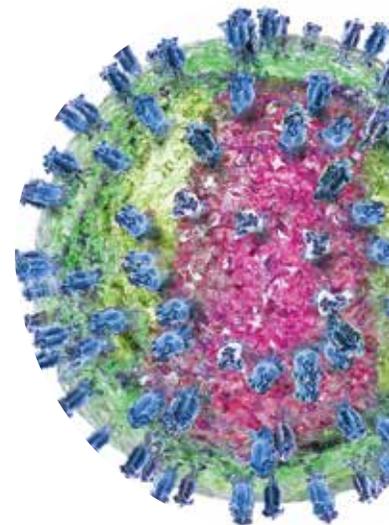
Study investigators were unable to say why the women who enrolled in VOICE didn’t stick to their assigned regimens, but they hope a sub-analysis now underway will be able to shed light on some of these behavioral questions.

In a related PrEP talk, Chasity Andrews of the Aaron Diamond AIDS Research Center in New York reported results of a study of 16 Indian rhesus macaques that suggests an alternative to daily PrEP. The study evaluated the ability of GSK744, a long-lasting second generation drug, to protect monkeys from SHIV. GSK744 belongs to a family of drugs called strand transfer inhibitors, which block integrase, the protein HIV uses to insert its genetic material into chromosomes.

Clinical studies have suggested that a single intramuscular injection of GSK744 can be detected for up to 48 weeks, which makes it an attractive option for both HIV treatment and PrEP. Andrews said eight monkeys who were injected intramuscularly with a long-acting form of GSK744 twice over a four week period were protected against repeat intrarectal SHIV challenge. All eight control monkeys became infected after a median of two rectal challenges. Andrews said the results suggest that GSK744, when injected quarterly or monthly, might be an effective alternative to daily oral PrEP or topical PrEP.

Meanwhile, an animal study led by the US Centers for Disease Control and Prevention in Atlanta found, for the first time, that a tenofovir intravaginal ring protected all six female pigtailed rhesus macaques following repeat low dose vaginal challenge by SHIV 162p3. By comparison, 11 of 12 control animals became infected after a median of four exposures. The treated animals remained fully protected after 16 weekly challenges with the SHIV strain. The polyurethane reservoir ring containing 120 mg of TDF delivered, on average, 2.3 mg/day *in vitro* in macaques, resulting in high tissue concentrations of the medication.

These studies have yet to be replicated in human trials. But they may well foreshadow where PrEP research is heading in the near future: the development of products and strategies to solve the seemingly intractable problem of adherence. —Regina McEneaney



# *The Antibody* RACE

This year's Keystone symposium on HIV vaccines, held jointly with B-cell immunologists, focused unapologetically on antibodies against HIV

By Andreas von Bubnoff

Organizers of the annual Keystone symposium on HIV vaccines like to mix things up a bit, combining their gatherings with those of other research tribes. They do this ostensibly to encourage the exchange of ideas—though to what extent that actually occurs is anybody's guess. But this year, it wasn't much of a stretch: The co-hosts of their latest meeting, which was held in Keystone, Colorado, from Feb. 10-15, were folks concerned primarily with B-cell development and function.

For observers of the field, this should come as no surprise, given the many antibody-related advances the field has seen in recent years. “We now have an increasingly detailed map of the vulnerable sites on HIV Envelope due to an array of newly discovered broadly neutralizing antibodies and the identification of their targets,” explained Georgia Tomaras of the Duke University Medical Center, who co-organized the HIV vaccines track of the conference. Capitalizing on that information, she said, is a priority of the field—sufficient reason, at any rate, to have the B-cell folks over for the conference.

And, indeed, antibody aficionados were probably not among the disappointed in Colorado that week.

## **Toward passive protection**

Now that dozens of broadly neutralizing antibodies (bNAbs) have been isolated from HIV-infected people, the next logical question is whether their presence in uninfected people prevents infection. One approach to finding out is to devise vaccine candidates that might coax a naïve immune system to make anti-HIV bNAbs of its own. Another is passive immunization. The advantage of the latter

approach is that researchers won't have to cross their fingers and wait for the immune systems of vaccine recipients to go through the long process of affinity maturation that leads to the cherished bNAb.

Besides, a successful passive immunization trial would provide proof of principle for an antibody-based vaccine and perhaps some guidance on how much neutralization activity might be needed for protection from HIV, said Barney Graham of the National Institute of Allergy and Infectious Diseases (NIAID). “Many of our licensed vaccines [such as measles vaccine] were preceded by demonstrations that passive immunization is effective in preventing or diminishing disease,” he said.

Graham, who gave an overview of passive immunization projects at the NIAID's Vaccine Research Center (VRC), said that VRC researchers have recently shown that passive immunization with VRC01, one of the broadest and most potent HIV-specific bNAbs, can protect rhesus macaques from challenge with SIV/HIV hybrid (SHIV) viruses. The VRC researchers injected human VRC01 intravenously or subcutaneously into animals. They then challenged the animals two days later, vaginally or rectally, with SHIV SF162P3—which is relatively difficult to neutralize—or the more easily neutralized SHIV BAL.

The result: At a concentration of about 40 micrograms per milliliter of blood, VRC01 protected all animals from challenge with SHIV SF162P3. Concentrations of only five micrograms per ml, meanwhile, sufficed to protect the animals from SHIV BAL. The higher concentrations, Graham said, are routinely achieved in

transfusions of a prophylactic antibody named Palivizumab, which is given to infants to prevent infection with respiratory syncytial virus.

Graham said VRC01 is currently the only bNAb manufactured at a GMP grade, and the VRC plans to apply for FDA approval to begin a Phase I passive immunization trial using human VRC01 in adults at the NIH clinic in Bethesda in the fall of this year. “VRC[01] is potent enough and manufacturable enough and deliverable enough to answer the question whether an antibody with a certain level of neutralizing activity can protect” people from HIV, Graham said.

One limitation of the VRC’s macaque experiments using human VRC01 is that human antibodies only have a half-life of 4-5 days in the animals, since the animals launch an immune response against the human antibodies. That’s why the VRC researchers waited only two days after infusing the antibody before they challenged the macaques with virus.

To reduce antihuman immune responses, VRC researcher Kevin Saunders grafted the human part of the antigen-binding region of VRC01 onto a monkey antibody. This increased the half-life of the molecule to about nine days. A one-time infusion with the “simianized” VRC01 protected the animals from infection for two weeks. While the half-life of VRC01 in humans isn’t known, the half-lives of antibodies of this type are typically about 21-24 days in the human body. Graham said this suggests that VRC01 will probably have to be given once a month to volunteers in the proposed trials.

While VRC01 is potent enough to get meaningful results in human trials, VRC researchers have isolated another bNAb called VRC07 from the same donor who provided VRC01. This antibody is about 2.5 times more potent and neutralizes 93% of circulating HIV variants, as opposed to VRC01’s breadth of 91%. They have also made dozens of modifications to several bNAbs that make them up to 15 times more potent, or increase their half-lives, Graham said.

But these modifications come at a price: The “vast majority,” said Graham, render the antibodies autoreactive—meaning that they are likely to bind to the body’s own tissues. Because the immune system normally eliminates B cells that make such antibodies, naturally occurring bNAbs are less likely to be autoreactive.

There are exceptions, however: *in vitro* studies suggest, for example, that two bNAbs—b12 and 4E10—might be autoreactive. But *in vitro* exper-

iments may not always reflect the *in vivo* situation, said David Nemazee of The Scripps Research Institute, adding that the real test of whether the autoreactivity of an antibody matters *in vivo* is to study whether these antibodies are actually likely to be eliminated by the immune system.

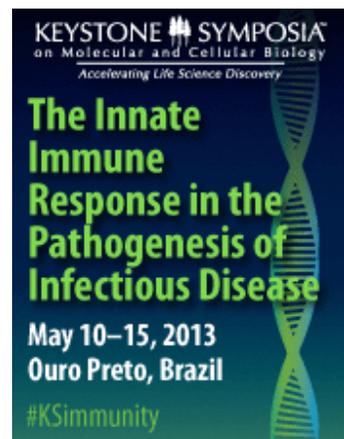
To test this, Nemazee and colleagues made transgenic mice whose B cells either produce b12, which targets the CD4 binding site of Env, or 4E10, which targets the part of Env on the HIV surface that’s closest to the viral membrane, known as the membrane proximal external region (MPER). They found that most of the 4E10-producing B cells were indeed eliminated in the mice. However, this was not the case for b12, suggesting that *in vitro* tests of autoreactivity don’t always reflect the situation *in vivo*. Two years ago, Barton Haynes and colleagues also used transgenic mice to show that the MPER-specific bNAb 2F5 is eliminated in mice. Together, these findings suggest that the MPER part of Env might have a tendency to induce autoreactive bNAbs (see *Research Briefs, IAVI Report*, Sep.-Oct. 2011).

## Inducing the elusive bNAb

The alternative to directly infusing bNAbs into people is, of course, to develop a vaccine that coaxes the immune system to make them itself. That’s no easy task. bNAbs are only found in a fraction of HIV-infected people, and they take years to develop because they need to mature and mutate away from their germline precursors through a process called affinity maturation. Unfortunately, the native HIV Envelope protein binds these germline precursors only weakly or not at all, while it binds strongly to the final, mature bNAbs. A vaccine that contains the naturally occurring Envelope, or parts of it, as an immunogen would not be expected to reliably bind and activate B cells in uninfected people. It would therefore fail to kick-start the affinity maturation process that gives rise to bNAbs.

To address this problem, researchers are developing versions of HIV Envelope, or artificial immunogens that are similar to Envelope, that can bind the germline precursors of bNAbs and so initiate the affinity maturation process. Later immunizations could then use immunogens that resemble more natural versions of Env that can bind the more mature bNAbs and so guide the affinity maturation towards the most potent versions of bNAbs.

At the meeting, Bill Schief of The Scripps Research Institute reported that his team has constructed artificial immunogens bearing versions of HIV Env sites recognized by HIV-specific bNAbs. To present these “engineered outer



domains” (eODs) to the immune system, the researchers made a virus-like nanoparticle that can present 60 eODs on its surface. Researchers believe that the repetitive display of an antigen on the surface of a virus-like particle is an important feature of successful vaccines (see *Taking the Gritty Approach, IAVI Report*, Nov.-Dec. 2012).

Schief said his team has developed several different eODs. One of them, eOD17, resembles the CD4 binding site on HIV Env. It binds strongly to CD4-binding-site specific bNAbs and activates mature B cells that have a VRC01-like B-cell receptor. Because it binds much less strongly to non-neutralizing antibodies, it is also less likely to induce them. Other eODs have been engineered to resemble the CD4 binding site of five different HIV clades, so that they might induce neutraliz-

ing antibodies that are broader in their specificity.

Schief’s team also developed an eOD named eOD-GT6 that can bind mature VRC01-like bNAbs as well as their (inferred) germline versions (*Science* 2013, doi: 10.1126/science.1234150). When presented as a 60-mer on a nanoparticle, the immunogen potentially activated germline B cells in laboratory studies. Because the immunogen binds to both germline and mature versions of VRC01-like bNAbs, it could in theory be used as a prime to kick-start the affinity maturation process, and then possibly again as a boost to guide the process towards the more mature bNAbs (which it binds more tightly). Next, the Schief team will use their eOD-GT6 nanoparticle immunogen to immunize rhesus macaques.

Andrew McGuire of the non-profit research institute Seattle BioMed reported that he and his colleagues have developed versions of Env that can also bind putative germline ancestors of bNAbs (*J. Exp. Med.* 2013, doi: 10.1084/jem.20122824). He achieved this by changing just one amino acid in the protein to prevent the addition of a sugar that is ordinarily added to that site. McGuire said the resulting Env protein can bind germline versions of the bNAbs VRC01 and NIH 45-46, which were obtained from the same donor. The modified Env can also activate B cells that express the B-cell receptors of the germline precursors of those bNAbs. This suggests it might induce the production of bNAb precursors if used as an immunogen.

McGuire isn’t the first to show that removing sugar groups enables Env to bind germline versions of bNAbs. Two years ago, Haynes and colleagues showed that removing part of the sugar groups enabled Env to bind to the germline versions of the MPER-specific bNAbs 2F5 and 4E10 (see *Research Briefs, IAVI Report*, Sep.-Oct. 2011).

One challenge when using Env for immunizations is that the protein is highly unstable. It even vibrates, according to Quentin Sattentau from the University of Oxford. As a result, it’s difficult to induce protective immune responses using the protein—its constant instability distracts the immune system by offering up a parade of changing structural targets. “The B cells have trouble hitting the right epitopes because it’s like jelly,” Sattentau said.

Schief and his colleagues address this problem by creating stabilized artificial immunogens. Sattentau, on the other hand, stabilizes Env by treating it with glutaraldehyde (GLA), a chemical that crosslinks certain amino acids and so “fixes” proteins in a relatively static structure. When Sattentau and his colleagues treated soluble gp140 Env trimer with GLA, the fixed Env was less

## Understanding Adjuvants

The vaccine used in RV144 contained alum, an adjuvant that has been around for decades and is used even today in most licensed vaccines. It significantly boosts antibody responses, but is less effective in inducing cellular immune responses. To find better adjuvants for future HIV vaccine candidates, researchers are studying the mechanism by which alum and other adjuvants enhance the effects of vaccines.

But surprisingly little is known about how adjuvants, including the old standby alum, exert their effects. That includes their *in vivo* effects in the first 24-72 hours after immunization. To find out what happens in those early stages, Karin Loré from the Karolinska Institutet, who is currently a visiting scientist at the Vaccine Research Center (VRC), injected different muscles of rhesus macaques with HIV Env protein, combined with three different adjuvants: alum alone; alum combined with the toll like receptor agonist 7 (alum/TLR7); and the oil-based adjuvant MF59. Loré said she chose to study the mechanisms for these adjuvants because studies by VRC researcher Robert Seder in macaques suggest that both alum/TLR7 and MF59 elicit better B- and T-cell responses than alum alone. MF59 is an adjuvant that has been licensed in flu vaccines in Europe and other countries outside the US.

Peering into the injected muscles and lymph nodes that drain the injection sites 24 hours later, she found that all adjuvants caused a migration of more immune cells into the draining lymph nodes than did no adjuvant.

But there were differences: Alum/TLR7 induced type I interferon alpha, which inhibits viral replication and increases antigen presentation by dendritic cells (DCs) to CD4<sup>+</sup> and CD8<sup>+</sup> T cells. In contrast, MF59 had less of an effect on DCs, but caused a migration of neutrophils into the draining lymph nodes.

In additional experiments, Loré showed that neutrophils taken from the injected animals’ lymph nodes could act as antigen presenting cells: They presented Env antigens to activate memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells, albeit at lower levels than DCs.

This, Loré said, suggests that MF59 might make up for its deficiencies in antigen presentation by DCs by inducing antigen-presenting neutrophil migration into the lymph nodes. “We found,” she said, “that although DCs are superior at presentation, as expected, neutrophils can do some of the job. The fact that [neutrophils] are so frequent [with MF59] makes you wonder if what they lack in potency they make up for in numbers.”

How this translates into vaccine efficacy remains to be seen. But, for now, Loré’s experiments suggest that alum alone may not be the best adjuvant to get responses that protect against HIV. —AvB

likely to fall apart than untreated Env. What's more, the GLA-treated Env bound as well to bNAbs such as VRC01 as the untreated Env, indicating that the GLA treatment hadn't changed the bNAb binding sites. In fact, the fixed Env actually bound weakly neutralizing antibodies less well than the unfixed Env, suggesting that it offers fewer distracting targets to the immune system than the "jelly-like" unfixed version.

In collaboration with IAVI, Sattentau and colleagues then used the fixed Envs to immunize rabbits. After four immunizations, there were more neutralizing antibodies in the animals immunized with the fixed Env than in those injected with the normal protein. Further, the modified Env elicited antibodies against the CD4 binding site, and to exposed parts that are normally quite flexible, such as the variable regions V1 and V2. In contrast, the unfixed Env elicited antibodies to Env regions that are normally hidden, consistent with the view that the unfixed Env is less stable. Next, Sattentau plans to immunize macaques with fixed Env trimers.

Researchers are also trying to better understand what factors are important for the induction and maturation of bNAbs. These certainly include T follicular helper (Tfh) cells, a type of CD4<sup>+</sup> T helper cell that interacts with B cells in the germinal center of lymph nodes and is thought to be central to affinity maturation. Analysis of Tfh cells might therefore help in assessing how well a vaccine induces bNAbs. While it is not possible to routinely do biopsies to isolate Tfh cells from lymph nodes, researchers have recently identified markers that can be used to track them in blood.

Using such markers, Hendrik Streeck, who is now at the US Military HIV Research Program, isolated Tfh cells from the blood of HIV-infected people who make bNAbs and from those who do not. When he cultured the Tfh cells from the former with naïve B cells, he found that the Tfh cells were better at inducing antibody-producing plasma cells than their counterparts isolated from folks who do not make bNAbs.

Streeck also found that IL21, a cytokine made by Tfh cells, was essential to this capability. In other words, it appears that Tfh cells from people with bNAbs are better at inducing plasma cells, and that IL21 might be involved in this process.

### **RV144: the gift that keeps on giving**

The analysis of plasma samples collected in the RV144 HIV vaccine trial—the only one so far to have come up with even a trace of a positive result—suggests that IgG antibodies to the V1 and

V2 loops of Env were associated with the modest protection afforded by the regimen evaluated.

To better understand what class of IgG antibodies those were, Tomaras and colleagues compared plasma samples from RV144 vaccine recipients with samples from an unsuccessful trial called VAX003. In VAX003, vaccine recipients didn't receive the pox vector prime that was used in RV144, but did receive the HIV gp120 immunization that was used as a boost in that trial. Given that RV144 detected protection from HIV and VAX003 didn't, Tomaras was looking for any immune responses that were better in RV144 than in VAX003. Such responses, she reasoned, would give clues about which immune responses can protect from HIV infection.

Tomaras and colleagues found that the RV144 samples had higher levels of an IgG subclass called IgG3 that was specific to V1/V2 of Env. This suggested that IgG3 antibodies might be responsible for the IgG-mediated protection observed in RV144. To test this hypothesis, Tomaras and colleagues reanalyzed plasma samples from 41 RV144 vaccine recipients who got infected and 205 vaccine recipients who did not. The samples in question were taken just before the first vaccination and two weeks after the final vaccination. Their analysis revealed that protected vaccine recipients indeed had higher V1/V2-specific IgG3 responses than unprotected vaccine recipients.

IgG3 antibodies are known for their ability to recruit antiviral effector cells by binding to the Fc receptor of such cells through their Fc portion. Therefore, the IgG3 involvement in protection suggests that such Fc receptor-mediated mechanisms might have played a role in protection in RV144.

One example for such a mechanism is antibody-dependent cellular cytotoxicity (ADCC), in which antibodies that are bound to an HIV-infected cell recruit innate immune cells (such as natural killer [NK] cells) that then kill the infected cell. This may explain another, rather surprising, finding from the analysis of RV144 samples—that blood levels of IgA antibodies specific to a part of Env called C1 were associated with greater risk for HIV infection in vaccine recipients: Tomaras reported that IgA antibodies might have blocked IgG-mediated ADCC. To show this, she and her colleagues isolated monoclonal C1-specific IgA and IgG antibodies from the same vaccine recipient and found that, at least *in vitro*, these C1-specific IgA antibodies kept the C1-specific IgG antibodies from binding to their target. As a result, the IgG antibodies couldn't activate the NK cells required for ADCC.

*Continued on page 19*

# Research BRIEFS

## Neutralizing HIV Tat-specific antibodies might be involved in protection

When talk turns to the kind of neutralizing antibodies that can prevent HIV infection, it always revolves around one and just one HIV protein: the Envelope (Env) protein that forms the viral spike. That's because Env is thought to be the only protein HIV carries on its surface that is exposed to antibody targeting.

But a recent study led by Ruth Ruprecht at Harvard Medical School suggests that antibodies to the HIV protein Tat, which jumpstarts the expression of HIV genes and is secreted by infected cells, might also be involved in protection (*J. Virol.* 2013, doi:10.1128/JVI.02888-12).

The researchers studied the antibody responses of rhesus macaques they had immunized by intramuscular injection of a vaccine that contained the SIV proteins Gag and Pol, and HIV-1 Env and Tat. They then challenged the animals with an SIV/HIV hybrid virus (SHIV) that contained these same proteins, except for Env which had been derived from a different HIV strain. After five low-dose rectal challenges, 16 of 17 unvaccinated animals got infected, while four of the 12 vaccinated animals remained virus free.

### How can you prevent infection by targeting a protein that the virus doesn't carry with it?

— Robert Gallo

In a previous study of the same animals, they had reported that the protected animals differed from the ones that were not protected in that they had higher titers of neutralizing antibodies as well as cellular immune responses to Gag and Tat (*PLoS One* 6, e22010, 2011).

In the new study, Ruprecht and colleagues used an unbiased approach to see how the antibody repertoires in vaccinated animals that were protected differed from vaccinated monkeys that were infected: They mixed the serum of one protected animal with a library of phages that carried billions of different random peptides on their surface. They then discarded phages that bound to antibodies from an unprotected vaccinated animal. By repeating these steps, they isolated phages that only bound to the antigen binding sites of antibodies in the serum of the protected, but not the unprotected, animal.

As expected, they found that some of these phages carried Env-related peptides. But they also found peptides that were identical to parts of HIV Tat that are targeted by antibodies that neutralize Tat activity; what's more, they found high levels of antibodies that bind to these peptides in all but one of the protected vaccinated animals, but not in the unprotected vaccinated

animals. This suggests that neutralizing antibodies to Tat were in part responsible for the protection of these macaques.

One possible mechanism of protection, Ruprecht says, comes from a study published late last year that was led by Barbara Ensoli at the National AIDS Center in Rome, Italy (*PLoS One* 7, e48781, 2012). Ensoli and colleagues reported that HIV particles carry Tat molecules on their Env spikes. These Env-bound Tat molecules enable HIV to use an unusual receptor called integrin to infect dendritic cells, which can then transmit the virus to its main target cells, the CD4<sup>+</sup> T cells. What's more, when Tat binds Env, it appears to shield Env from neutralization by Env-specific antibodies. Tat antibodies, the researchers found, could prevent this by keeping Tat from binding to Env, and could neutralize HIV by binding to the Tat-Env complex on the virus.

For Ruprecht, the message for vaccine development is clear: Include Tat in a vaccine, she says, adding that the vaccine she used to immunize the macaques also induced cellular immune responses to Tat. "You get two for one," she says.

Michael Murphey-Corb at the University of Pittsburgh, who heard Ruprecht present her study a few months ago, agrees that it makes sense to include Tat in a vaccine. She is especially impressed with the phage technology Ruprecht and colleagues used in the study. "It's unbiased and that's what I like about it," she says.

But Robert Gallo—who with Ensoli discovered in 1990 that Tat is released from infected cells—says Ensoli's recent results don't convince him that Tat is important in preventing infection in a vaccine. "How can you prevent infection by targeting a protein that the virus doesn't carry with it [as a bona fide surface protein]?" asks Gallo. "You only get Tat after the cell was infected."

Gallo, who is at the Institute of Human Virology of the University of Maryland School of Medicine, says he found that Tat can have toxic effects on cells, and that he has unpublished results he presented at meetings that a candidate vaccine that's based on modified Env protein is effective in monkeys, but loses that effectiveness once Tat is added. "When we add Tat, we take away all protection," he says, "because it activates T cells that are targets for infection and you get more animals infected easier."

This isn't the first time Gallo's observations differ from Ensoli's. More than ten years ago, Gallo and colleagues found that in rhesus macaques, vaccination with a form of Tat that was modified to make it less toxic only led to a modest reduction in virus titers after challenge (*Proc. Natl. Acad. Sci.* 97, 3515, 2000). That was in contrast to a 1999 study by Ensoli and colleagues, who reported that vaccination with Tat protein could protect cynomolgus macaques from SHIV infection in some cases and reduce the virus to undetectable levels in others (*Nat. Med.* 5, 643, 1999). —*Andreas von Bubnoff*

# Vaccine BRIEFS

## Rallying for research

Volunteers from more than 175 medical research organizations planned to rally April 8 in Washington, D.C., to urge lawmakers to shield public health and scientific research from sweeping spending cuts that are likely to cripple several government programs in the US. As it happens, that's two days before President Obama is scheduled to release his budget for fiscal year 2014.

As *IAVI Report* went to press, the plan was for the rally to start at 11 a.m. on the Carnegie Library Grounds of Mt. Vernon Square and run for a little more than an hour. Regan Hoffman, an AIDS activist and former editor of *POZ* magazine, was to be one of the speakers addressing the protestors, and several HIV/AIDS organizations, including The Foundation for AIDS Research (amfAR), HIV Medicine Association, AIDS United and AIDS Action Baltimore said they would be there as well. (For a full list of participants, visit [www.rallyformedicalresearch.org](http://www.rallyformedicalresearch.org); and for updates on the rally, visit the *IAVI Report* blog.)

Federal agencies are still shakily coming to grips with the automatic spending cuts—a.k.a. sequestration—which took effect March 1 and will indiscriminately slash US\$85 billion from government programs this year. Their bean-counters have been busy ever since, working out how to make ends meet: the US Federal Aviation Administration announced plans to shut down 146 of 516 air traffic control towers beginning April 7, and the National Parks Service is all set to close visitor's centers and rest rooms, furlough park police, and hire fewer seasonal workers this summer.

Researchers and service providers across the country, meanwhile, are bracing for changes to their grants and contracts from the US National Institutes of Health, which stands to lose about \$1.5 billion of its \$31 billion annual budget (see *IAVI Report* March 5, 2013 blog, *Sequester Overshadows Science*). According to the NIH, many researchers whose grants aren't dropped outright can expect painful cuts in funding, and financing for new grants and cooperative agreements will be extremely tight.

"The immediate impact is still being sorted out," said Chris

Collins, vice president and director of public policy at amfAR. "Over the long run, I am concerned that we are sleepwalking into an era of austerity. I think we all need to be speaking out about the importance of health investment right now."

The human cost could be considerable. In a policy brief assessing the potential impact of cuts in global HIV/AIDS funding, amfAR suggested that sequestration would deprive 273,000 HIV-infected people from low-income countries of access to life-prolonging antiretroviral therapy, and curtail antiviral drugs and services for 111,000 HIV-infected pregnant women, which in turn could lead to 21,000 infants being infected with HIV.

Since the cuts took effect, committees from both the US House of Representatives, which is controlled by the Republicans, and the US Senate, nominally controlled by the Democrats, have submitted competing budgets for FY2014. They offer vastly different visions for the country. The Republican bill slashes government spending by \$4.6 trillion over the next decade, including steep cuts in domestic programs and a 10% reduction in the federal workforce. The Senate version offers \$1.85 trillion in deficit reduction through a mix of spending cuts and revenue increases. The House bill assumes the cuts dealt by sequestration will stay, while the Senate bill rolls them back. "Obviously, there is much better news for health research in the Senate proposal," said Collins. "But we're really waiting to hear from the President."

The rally is aimed at both citizens and lawmakers, according to Collins. "This is an opportunity to say health research has tangible benefits for real people and there is great opportunity in the future to accomplish much more in terms of curing disease and advancing health. But to do this we can't divest from our investment in health research."

Heedless divestment, however, is the likely outcome in the absence of some coherent Congressional response—the probability of which is minute, regardless of what happens at Mt. Vernon Square. —*Regina McEnergy*

## New digs for The Ragon Institute

The Ragon Institute of Massachusetts General Hospital (MGH), the Massachusetts Institute of Technology (MIT), and Harvard has moved into a 216,000-square-foot building a few blocks from MIT, the Broad Institute, and the Whitehead Institute. Redevelopers recently transformed the building from a nine-story office facility into a 10-story laboratory and office complex. Along with The Ragon Institute, which occupies three-and-a-half floors, the building currently has three other tenants:

Epizyme, Warp Drive Bio LLC, and Aramco Services.

The Ragon Institute, a rather unusual collaboration of engineers, doctors, and biologists headed by MGH AIDS and TB researcher Bruce Walker, was created with a US\$100 million gift from Phillip "Terry" Ragon and his wife Susan. Its mission is to explore how the immune system combats disease, with an initial objective of developing an AIDS vaccine. Terry Ragon, founder of the Cambridge-based international software company InterSystems, became drawn to the

field of AIDS vaccines after meeting Walker and hearing about his work (see *In With The New...*, *IAVI Report*, Jan.-Feb. 2009).

The Ragon Institute's new space includes a vivarium and a Biosafety Level 3 lab, which will enable Ragon researchers to study

TB in conjunction with HIV for the first time. The new space also includes a private phlebotomy room, the 160-seat Mark and Lisa Schwartz Auditorium—named for another major Ragon donor—and a large flow cytometry lab. —*Regina McEnerly*

## First candidate HIV vaccine to employ Sendai vector poised for trials

The first Phase I trial to test the safety and immunogenicity of a novel Sendai vector-based vaccine candidate got underway in Rwanda on April 1 and is expected to begin soon in the UK and Kenya. The SeV-G (NP) vector, so named because it carries the HIV *gag* gene as its immunogen, will be evaluated in prime-boost combination with an adenovirus (Ad) serotype 35 vector candidate named Ad35-GRIN.

Derived from the Sendai virus—which belongs to the *Paramyxoviridae* family of viruses that includes the measles, canine distemper and human parainfluenza viruses (hPIV)—the SeV-G(NP) candidate retains the ability to replicate following vaccine delivery. Though Sendai causes a respiratory tract illness in rodents, it is not known to cause human disease.

This is only the fourth time that a replicating vector has been evaluated in clinical trials. A replicating vaccinia vector (based on the Tiantan strain) was tested in a Phase I trial in China several years ago (see *Raft of Results Energizes Researchers*, *IAVI Report*, Sep.-Oct. 2009), and a replicating measles vector was used in a Phase I study in Belgium. In addition, a vesicular stomatitis virus is being used in an ongoing study in the US.

The Ad35-GRIN candidate, meanwhile, has been evaluated in three clinical trials, and has been found to be safe and well tolerated so far. It carries as HIV immunogens the genes for HIV clade A *gag*, *reverse transcriptase*, *integrase*, and *nef*.

The double-blind, dose-escalation trial will be conducted in 64 healthy, HIV-uninfected individuals, with groups of 12 vaccine recipients and four placebo recipients randomized to four arms. The first group will receive a lower dose of SeV-G(NP), administered intranasally, followed by an intramuscular injection of the Ad35 viral vector vaccine candidate four months later. The second will receive a higher dose of SeV-G(NP) followed by Ad35 four months later. The third will receive the Ad35 candidate followed by SeV-G(NP) four months later, and the fourth will receive two intranasal administrations of SeV-G(NP) four months apart.

SeV-G (NP) was developed by the Japanese biotech DNAVEC, while the Ad35 viral vector candidate was developed by IAVI, which is sponsoring the Phase I trial.

In addition to testing the safety and tolerability of the vaccine regimens, the study will evaluate HIV-specific cellular and humoral immune responses in peripheral blood and SeV-specific neutralizing antibodies in all vaccine groups. It will also assess viral shedding in nasal swabs, saliva, urine, and plasma from volunteers to assess the vector's persistence.

Dagna Laufer, IAVI's Senior Director for Medical Affairs,

said another aim of the trial is to see how well intranasal immunization alone or in a prime-boost regimen with Ad35 induces systemic and mucosal immune responses. As such, researchers will be conducting extensive mucosal sampling to assess HIV-specific Gag antibodies in saliva, transudate (oral fluids that pass through membranes), and nasal, rectal, and vaginal secretions. They will also assay SeV binding antibodies in nasal secretions. The study will further assay such binding antibodies in the serum of all four arms. And among volunteers willing to undergo colo-rectal biopsies, researchers will look for cell-mediated immune responses induced by the vaccine regimen.

Because different vaccination routes elicit different mucosal responses, researchers will look for evidence that nasal immunization stimulates an immune response not only in the respiratory mucosa but in more distant mucosal sites, such as the vagina and gut as well.

Researchers previously evaluated the Sendai as a live, xenotropic vaccine candidate in a Phase I trial for hPIV. That vaccine candidate, which was also administered intranasally, was found to be immunogenic in three of the nine vaccinated volunteers and generally well-tolerated, despite suspected cross-reactive immunity thought to have been induced by previous exposure to hPIV (*Vaccine* 22, 3182, 2004). But this is the first time Sendai is being used in an AIDS vaccine trial.

One possible obstacle to using SeV vectors could arise from pre-existing immunity to hPIV type 1, which can elicit responses to SeV proteins. As a result, multiple immunizations might be required to achieve a persistent HIV-specific immune response. But an animal study conducted by Chinese researchers suggests another way around this obstacle. While the immunogenicity of SeV, when used alone, is generally not much better than that of a DNA vaccine candidate, the Chinese study found that using a combination of triple heterologous vectors—Sendai, adenovirus, and DNA—encoding *gag* induced broad and sustained high levels of Gag-specific immune responses in mice and monkeys (*Vaccine* 26, 6124, 2008).

In a separate study in animals, a prime-boost regimen that used combinations of replication-competent vaccinia LC16m8Δ (m8Δ) and SeV vectors expressing HIV-1 Env efficiently produced both Env-specific CD8<sup>+</sup> T cells and anti-Env antibodies, including neutralizing antibodies (*PLoS One* 7, e51633, 2012).

Which raises the question: Would a SeV candidate be better off containing both Env and Gag inserts? We might soon have an answer: scientists are already developing second-generation SeV candidates encoding proteins more likely to engage both arms of the adaptive immune response. —*Regina McEnerly*

## World Health Organization streamlining HIV guidelines

Ongoing discussions to consolidate and streamline the World Health Organization's (WHO) hefty and sometimes confusing HIV treatment and prevention guidelines could boost the number of people in low- and middle-income countries eligible for antiretroviral treatment (ART) from 15 million to at least 23 million.

The revised guidelines are likely to recommend that HIV-infected individuals become eligible for ART when they have fewer than 500 CD4<sup>+</sup> T cells per microliter of blood. But the WHO is also expected to temper this dramatic shift by suggesting that those with fewer than 350 CD4<sup>+</sup> T cells per microliter of blood be given priority.

This expected change is part of a larger effort by the WHO to consolidate all aspects of ART for treatment and prevention for all age groups and populations, according to Gottfried Hirschall, director of the WHO's HIV Department. The document should simplify and reduce the number of first-line and second-line drug regimens. It will also seek to provide guidance to countries on how to link people to testing and treatment and retain those being treated in the health care system.

About a year ago, the WHO began recommending that HIV-

infected individuals in serodiscordant relationships be offered ART when they have CD4<sup>+</sup> T-cell counts under 350. The recommendations stemmed from compelling data obtained from the HPTN052 trial involving 1,763 serodiscordant couples. That study demonstrated that earlier ARV administration can dramatically reduce HIV transmission (see *Treatment as Prevention, IAVI Report*, Jul.-Aug. 2011). The WHO also issued revised guidelines on the prevention of mother-to-child transmission. But the authors of those guidelines ultimately omitted mention of any review of current data on the preventive benefits of ART in populations other than heterosexual discordant couples.

Now, with evidence suggesting that early treatment improves prognoses and reduces transmission, many advocates and some researchers are calling for the expansion of ART to everyone who is eligible and willing to adhere to prescribed regimens (see *Is It Ever Too Early?*, *IAVI Report*, Sep.-Oct. 2012).

Hirschall said the plan is to release the consolidated guidelines at the upcoming International AIDS Society meeting this summer in Kuala Lumpur, Malaysia. —*Regina McEnery*

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*Continued from page 15*

### Antibodies and mother-to-child transmission

Even though antiretroviral therapy (ART) reduces the incidence of HIV transmission from mother to infants to 1-2%, more than 300,000 mothers transmit HIV to their infants each year. About half of these transmissions are believed to occur through breast milk.

But even without ART, 90% of children are protected from HIV transmission from their mothers. One reason for this, a recent study suggests, is that women who don't transmit HIV have antibodies in their breast milk that can better induce ADCC (*PLoS Pathog.* 8 e1002739, 2012). "It's not just how much antibody you have in breast milk that's specific for HIV," said Sallie Permar of Duke University Medical Center. "It's really the function of the antibody that's in breast milk [that determines] whether you will be better protected."

To learn how to reduce mother-to-child transmission even further, Permar is studying why African green monkeys (AGMs), which are natural hosts of SIV infection, rarely transmit SIV to their babies through breast milk, while rhesus macaques, which aren't natural hosts of SIV infection, transmit it in most cases. Permar and colleagues induced lactation in chronically SIVmac251-infected rhesus macaques and in chronically SIVagm-infected AGMs, and studied their breast milk. While viral load, CD8<sup>+</sup> T-cell responses, and levels of Env binding antibodies were similar in the two species, only breast milk from the AGMs contained IgG antibodies that could neutralize the difficult-to-neutralize founder virus that initially infected the animal.

HIV-infected human mothers also have HIV-specific neutralizing antibodies (NAbs) in their breast milk, but these can only neutralize easy-to-neutralize HIV variants, Permar said. Permar therefore wants to develop a vaccine that can induce more potent NAbs similar to what she observed in the AGMs to further reduce mother-to-child transmis-

sion in humans. She and her colleagues have immunized lactating rhesus macaques with different types of vaccines to see if they can induce the kinds of virus-specific NAbs she has observed in AGMs. The results of the study, which have been submitted for publication, are promising, Permar said: Some of the vaccination strategies induced antibodies that can neutralize viruses that are relatively resistant to neutralization.

### A solution that sticks

Much of the research reported at the meeting focused on neutralizing antibodies, but Olga Malykhina from Tom Hope's lab at Northwestern University said that even antibodies that do not neutralize HIV might be able to prevent infection. She reported that many endogenous IgG antibodies bind to vaginal and cervicovaginal mucus, probably through their Fc receptor end. The mucus binds the antibodies and keeps them from moving.

If such mucus-binding antibodies bind HIV, they should be able to prevent sexually transmitted infection, Malykhina ventured. "As long as [the virus] is trapped, it could be expelled from the genital tract" along with the mucus, she said, adding that mucus naturally exudes out of the vagina. Such antibodies wouldn't need to neutralize HIV for this to happen, she said, because as long as they bind to both mucus and HIV, it wouldn't matter to what part of HIV they bind. Perhaps, she said, some of the protection observed in the RV144 trial was due to mucus-binding antibodies.

Now Malykhina and Hope want to better understand why some antibodies can bind mucus better than others. By applying this information, they say, it might be possible to develop a vaccine that preferentially induces certain types of HIV-binding IgG antibodies that bind mucus especially well. ■

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