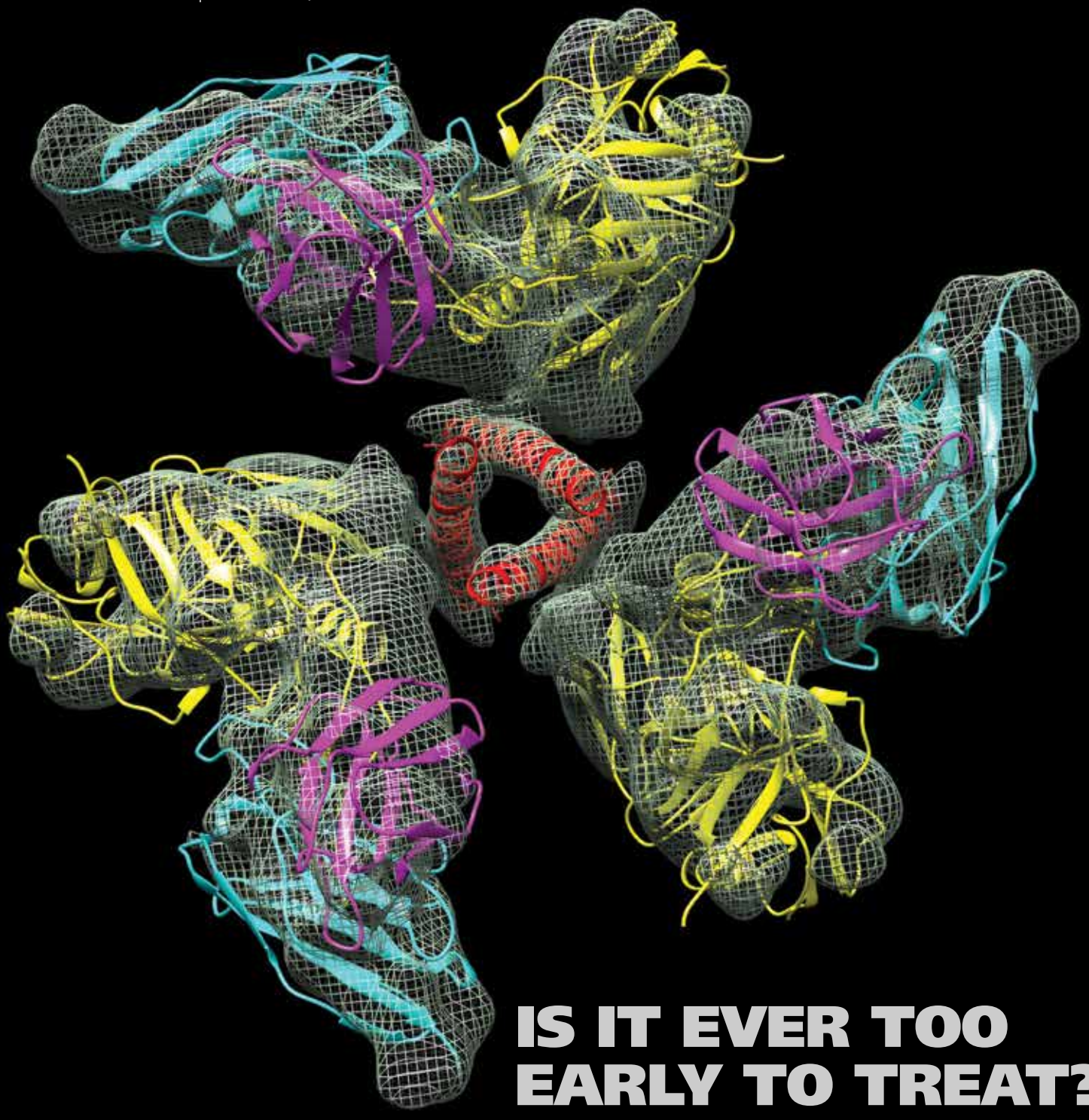
SEPTEMBER-OCTOBER 2012

IAVIReport

The Publication on AIDS Vaccine Research

WWW.IAVIREPORT.ORG | VOLUME 16, NUMBER 3



IS IT EVER TOO EARLY TO TREAT?

EDITOR'S LETTER

Let me begin by addressing the elephant in the room. And I don't mean the outcome of the general election.

As you've probably heard, we have had a rough few days in New York City, ever since Hurricane Sandy made landfall on October 29th, followed by a sizable nor'easter. The hurricane, which left a swathe of devastation along the coasts of New York and New Jersey, drove a tide of floodwater from the East River into the subbasement of the building that houses IAVI's headquarters—and the offices of *IAVI Report*. This happened just as we were getting ready to send the current issue off to our printer—which is why the magazine is coming to you a few days later than usual.

Our building, needless to say, is now closed and we've all been working from home. But we consider ourselves lucky. A good many people lost their homes, possessions, and livelihoods to the storm. Some have suffered the loss of loved ones. We extend our deepest sympathies to them, and urge our readers to do what they can to extend a helping hand to the thousands across the area who are today picking up the pieces of their lives.

On to this issue of *IAVI Report*. In the pages that follow you'll read one major article that covers a brewing debate over how quickly people infected with HIV should be offered antiretroviral therapy. It explores existing evidence that early treatment improves prognoses, alternative takes on that evidence, and studies that seek to test the disputed hypothesis. Another big story surveys major news out of the 2012 AIDS Vaccine conference, which was held in Boston in September and had a notable emphasis on broadly neutralizing antibodies to HIV.

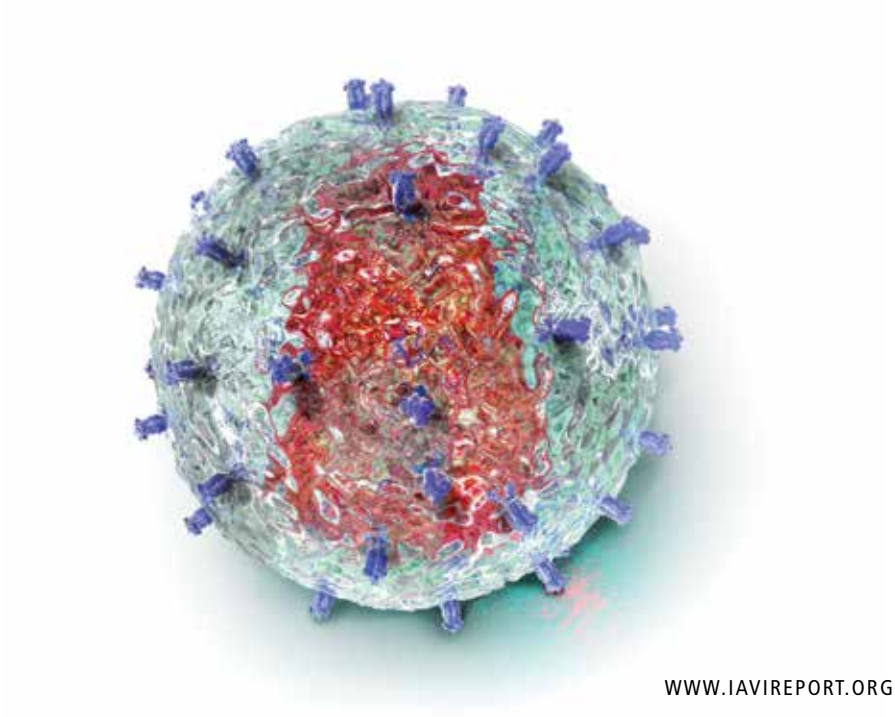
Our research briefs, meanwhile, focus on competing strategies for targeting South Africa's epidemic with expanded HIV testing and treatment—and what conflicting computational models say about the potential impact of each—and the induction of SIV control in rhesus macaques via immunization. Finally, we include in this issue an interview with MHRP director Nelson Michael.

You'll notice as you read through this issue that HIV researchers seem more optimistic than ever before about the prospects of a wide variety of HIV treatment and prevention strategies. We hope you will emerge from this issue of *IAVI Report* better informed about what fuels their optimism—and perhaps as inspired as they are these days.

– UNMESH KHER

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



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 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]

When the trimeric HIV Envelope (Env) spike binds its receptor on the target cell, it opens up and exposes an inner portion called gp41 that then drives fusion of the viral membrane with the target cell membrane. Recently, Sriram Subramaniam and colleagues at the National Cancer Institute of the NIH determined the detailed three-dimensional shape of this open state of Env (*PLoS Pathog.* **8**, e1002797, 2012). They first showed that if they mixed an antibody called 17b with a water-soluble version of the Env trimer that only contained the parts outside the viral membrane and had a disulfide bond between the gp41 and gp120 parts, they could stabilize Env in the open conformation. They then rapidly froze the complex of 17b and Env and determined its structure using cryo-electron microscopy. The image shows this structure of the opened Env trimer bound to 17b at 9 Å resolution (gray mesh), filled with the known X-ray structures of its components. The three exposed gp41 helices are in the center (protein backbone in red), surrounded by three copies of gp120 (N-terminal helix in yellow) bound to 17b (light chain: purple; heavy chain: blue). A similar version of this image appeared in *PLoS Pathog.* **8**, e1002797, 2012.

Is it Ever *TOO EARLY?*

Some say that with better ARVs available, it's time to treat all HIV-infected individuals immediately, and even argue that early treatment could help prime some people for a future functional cure. Others worry it may do more harm than good.

By Andreas von Bubnoff

"Time to hit HIV, early and hard"—that was the title of an editorial that appeared in 1995 in the *New England Journal of Medicine*. The author was David Ho, a researcher at the Aaron Diamond AIDS Research Center in New York City. Ho played a major role in developing combination antiretroviral therapy (cART, a.k.a. highly active ART, or HAART), which revolutionized AIDS care. "I remember it like it was yesterday," says Jens Lundgren, an infectious disease physician at the Copenhagen University Hospital. The essay, he recalls, appeared at a time when researchers were seeing the first cases of HIV-infected individuals improving from the therapy. "For the first time in my career, I saw a CD4[+ T] cell count increasing. The editorial was written in that atmosphere."

But it would take another 17 years before two expert panels—one convened by the US Department of Health and Human services (DHHS), the other called the International Antiviral Society (IAS)-USA—recommended for the first time that all HIV-infected individuals be treated, regardless of their CD4+ T-cell counts, as long as the patients were ready and willing to adhere to therapy. IAS-USA also recommended for the first time that anyone acutely infected with HIV should be offered ART, even in the absence of symptoms. In principle, the guidelines are relevant for all countries, says Melanie Thompson of the AIDS Research Consortium of Atlanta, who chaired the IAS-USA panel that wrote the 2012 recommendations. But their full implementation may not be possible in developing countries, due to limited resources (see *Research Briefs*, page 18).

One reason it took until this year for the expert panels' recommendations to catch up with Ho's editorial is that the risk of side effects, and of developing drug resistance to antiretrovirals (ARVs), was long viewed by many as too great to recommend early treatment for everyone, says Martin Markowitz, who is also at Aaron Diamond. But now the drugs have fewer side effects than ever and are so potent, easy to take, and diverse in their mechanisms that the risk of developing resistance to them has declined.

Further, Thompson says, a growing number of studies show that starting ART earlier has lasting benefits, enough to support treatment at all T-cell counts. For example, HPTN 052, a randomized trial in serodiscordant couples, showed that starting ART at CD4+ T-cell counts of 350-550 cells/µl is better than waiting until the count drops to 200-250 (*N. Engl. J. Med.* **365**, 493, 2011). Earlier treatment reduced HIV transmission by 96%, and the early starters had fewer AIDS-related illnesses. "[After] David Ho wrote that editorial, it took [17] years to show that treatment does prevent transmission," says Markowitz, referring to HPTN 052. The science, he says, had always suggested early treatment was the right way to go. "Now that the drugs have caught up with the science and the science has matured, it's a pretty simple argument."

What's more, research also suggests that treating people very early, in the first few weeks and months after infection, can prevent much of the initial destruction of the immune system and diminish the HIV reservoir. Some researchers even suggest that, if combined with therapeutic vaccines or drugs that target the reservoir, a very early start of ART could lead to a functional cure.

Still, some researchers are calling for more studies before treatment can be recommended for everyone. It's unclear, they say, whether lifelong ART is beneficial in the long run in people who start early,

as opposed to waiting to start until the CD4+ count has dropped to levels around 350. For now, the IAS-USA and the US DHHS guidelines are the only ones that recommend starting treatment regardless of CD4+ T-cell counts. Others, such as the European AIDS Clinical Society (EACS) guidelines, only recommend starting treatment below 350.

Therapy in the acute phase

A growing body of evidence suggests early treatment has its benefits. Markowitz, for example, recently reported that people who start ART about 50 days after infection have lower immune activation, suggesting that it might delay progress to disease (see *IAVI Report* online Special Feature article, *Cure Research: Marching on—but over uneven terrain*).

Others have found that early treatment reduces the size of the HIV reservoir. Perhaps the first evidence for this came from a 2005 study that showed less viral outgrowth in cultured, latently infected CD4+ T cells taken from patients who started ART within six months following infection than in such cells from patients who started ART during chronic infection (*J. Infect. Dis.* **191**, 1410, 2005).

A team led by Huldrych Günthard of the University Hospital Zurich, who was involved in that study, later showed that compared with patients who started ART during chronic infection, those who started therapy three to 15 weeks after infection had a roughly 10-fold smaller HIV DNA reservoir in their white blood cells (*PLoS One* **5**, e13310, 2010).

Steven Deeks and colleagues at the University of California, San Francisco, have made similar observations. They found a five-fold smaller reservoir in white blood cells taken from people who had started ART within six months after infection and were then treated for at least two years, compared with people who started ART later than two years after infection. What's more, Mathias Lichterfeld at Massachusetts General Hospital in Boston and his colleagues studied nine patients who started ART one to two months after infection, and then remained on treatment for 10-15 years. It was not possible to retrieve any replication-competent virus from the CD4+ T cells from many of these patients, even when using a large number of cells from their blood. Lichterfeld made similar observations in elite controllers.

Perhaps the largest study of early ART starters is being conducted by Jintanat Ananworanich and colleagues at the Thai Red Cross AIDS Research Center in Bangkok, the largest HIV testing clinic in Thailand. The researchers screened more than 50,000 patient samples to identify 77 patients between one and about four weeks after infection.

They found that the earlier treatment was started, the smaller the reservoir size in blood and colon six or 12 months later. In fact, after half a year of treatment, the 19 people who had started ART the earliest—one to two weeks after infection—had a reservoir size matching that of elite controllers. It was also about 10 times smaller than in people who started ART during chronic infection and were treated for five years. Intriguingly, it appears that the HIV DNA in these 19 patients had not integrated into the host's white blood cell genome, whereas patients who started ART just two weeks later than them did have integrated HIV DNA. "That shows that if you capture people really early, you may be able to block further integration," Ananworanich says.

Perhaps, Lichterfeld says, starting ART within the first week after infection can prevent the establishment of the reservoir. To test this idea, he says, investigators at the Ragon Institute are now setting up a study in Africa in which high-risk patients get tested for HIV RNA every week; those found to be positive will be put on ART immediately and followed to permit measurements of their viral reservoirs.

Path to a functional cure?

Some patients who start ART during acute infection seem to be able to control the virus after stopping therapy, suggesting that they may be functionally cured. At a meeting on cure research just before the International AIDS Conference earlier this year in Washington, D.C., Asier Sáez-Cirión, an assistant professor at the Institut Pasteur, reported that he and his colleagues have studied 14 such cases (see *IAVI Report* online Special Feature article, *Cure Research: Marching on—but over uneven terrain*).

Patients in this so-called VISCONTI cohort started therapy on average 39 days after infection. They were identified and recruited by researchers, who searched hospitals across France for patients who had been treated for at least a year before treatment interruption, and who subsequently controlled their viral load for at least a year. Although the search did not exclude people who started therapy during chronic infection, the researchers discovered that all 14 of the patients identified and recruited had started therapy during the acute phase of infection.

These were, in other words, post-treatment controllers and not elite controllers—those rare HIV-infected people who control the virus without any treatment at all. Indeed, Laurent Hocqueloux, an infectious disease doctor in Orléans,

France, who coordinates the studies of the VISCONTI cohort, says their HLA alleles differ from those of elite controllers. While elite controllers are more likely than most people to have HLA alleles such as B27 and B57 that somehow contribute to better control of the virus, the VISCONTI patients are less likely to have these alleles. They are, oddly enough, more likely to have the B35 allele, which is associated with poor control of viral load and faster progression to AIDS.

This, Hocqueloux says, could explain why 90% of the VISCONTI patients showed symptoms when they were acutely infected, a phenomenon that probably accounts for their early identification.

To get a better idea of how many people who start treatment early control viral load, the French researchers also searched thousands of cases in French hospital records for cases of post-treatment control. They found 74 patients who started treatment within six months after infection, were treated for at least a year, and then stopped treatment. Of those, about 15% were able to control infection for two years after treatment was stopped—a much higher percentage than the roughly 0.5% of elite controllers in the general population. To Hocqueloux, this suggests that early treatment is the major reason the VISCONTI patients can control viral load after interrupting treatment. He says he is now looking for markers that can predict which patients can become post-treatment controllers.

Not everyone is convinced. Günthard says that he too has seen a few patients who controlled viral load after starting treatment early and then interrupting it about a year and a half later, "but we didn't make a big story out of it." He doesn't think the effect is necessarily due to the early start of treatment; it's unclear, he points out, what would have happened if they hadn't been treated early or if they hadn't been treated at all. And even if post-treatment controllers differ from elite controllers, Günthard says, it's possible that they control viral load by unknown mechanisms that differ from elite control but are also unrelated to the early start of therapy. For example, he says, the effect could be due to differences in the viruses these people are infected with. He has found that viral differences can affect the viral load even more than differences in HLA alleles.

The exact mechanism of post-treatment control, if it's real, is indeed unclear. Sáez-Cirión recently reported that the VISCONTI volunteers have a smaller viral reservoir than people who start therapy later, during chronic infection. In some, the reservoir even seems to be shrinking. Charline Bacchus of the Pitié-Salpêtrière Hospital in Paris recently reported that one possible explanation for this is that the reservoir consists of an unusually small fraction of long-lived cells (see *IAVI Report* online Special Feature article, *Cure Research: Marching on—but over uneven terrain*).

Yet a small reservoir alone isn't sufficient for viral control, Hocqueloux says. Even people with extremely small reservoirs can't always control the virus without treatment. He and his colleagues are therefore looking for other explanations. One possibility, he says, is that early starters have healthier immune systems. Hocqueloux says he has some evidence that that could be the case.

Consistent with that, Ananworanich and colleagues found that if ART is started within the first few weeks after infection, just one year of treatment can reconstitute $CD4^+$ T cells to almost normal levels in the blood and the gut. This usually does not happen in people who start ART later, during chronic infection, she says.

Another effect of early ART is that it slows viral evolution by nearly halting HIV replication. Sarah Palmer and colleagues recently reported that individuals who started ART during acute infection have a less diverse viral population (see *Stalking HIV's Sleeper Cells*, *IAVI Report*, Mar.-Apr. 2012). This, Hocqueloux says, could make it easier for their immune systems to keep the virus in check. "Perhaps the preserved immune system and the genetic restriction of the virus together lead to control," he says. He plans to sequence viruses in the VISCONTI patients to see if theirs are less diverse as well.

Meanwhile, a team led by Christine Rouzioux from the University Paris Descartes has started enrolling patients in a trial called OPTIPRIM, designed to explore the induction of post-treatment control. All 90 trial participants are to start ART within 10 weeks after infection and are randomly assigned to one of two groups. One will get ART with a traditional three-drug ART regimen similar to the one used by the patients in the VISCONTI cohort. The other will get a more aggressive five-drug regimen that additionally includes the CCR5 inhibitor maraviroc and the integrase inhibitor raltegravir. The treatments will be stopped, with careful monitoring, after two years and the researchers will check whether the participants can control viral load. Any who fail to do so will restart treatment immediately.

Rouzioux and colleagues hope that the more effective five-drug regimen will induce control in a larger fraction of post-treatment controllers than the three-drug regimen, and result in a

larger reduction of the reservoir size. They will try to identify biomarkers that are associated with control. Rouzioux says they will also study patients who can't control the virus to see which ART regimen leads to a longer delay in viral rebound, and which better preserves immune responses and reduces immune activation.

Should the five-drug regimen create a significant proportion of post-treatment controllers, early ART followed by closely monitored treatment interruption to check for post-treatment control could even someday become standard clinical practice, Rouzioux says.

"No one in the clinic ever wants to stop therapy these days," says Deeks. "In general, once we start people on therapy, we never stop unless we have to." However, he adds, "if a mechanism for post-treatment control can be identified, and a biomarker that predicts outcome, then it is possible some people who are potentially destined to do well can stop drugs." In addition, he says, reducing the size of the reservoir in patients who start ART during acute infection might make a future cure more feasible for such patients.

To test this idea, Ananworanich and colleagues are already planning to combine early ART with other treatments that boost the immune system or target the reservoir to see if that might result in a functional cure. They will, she says, assign volunteers randomly either to an early three-drug or to a five-drug regimen and then interrupt treatment. Next, they will check if some can control viral load either without treatment, or after treatment with therapeutic HIV vaccines or drugs such as SAHA that activate the HIV reservoir. "We feel that these patients have the highest chance of achieving functional cure because they have such [a] low reservoir and their immune system is likely intact," Ananworanich says. "The early treatment is not the whole answer. It's just to get them to a stage that they have very little virus [and a] good immune system and then test another strategy."

A need for more evidence?

Most data that support starting ART during acute infection come from observational studies, not randomized trials. One reason, Thompson says, is that it's difficult to find enough acutely infected patients for large randomized trials and follow them long enough to see clinical effects such as diseases or mortality. Only a small percentage of all HIV-infected people are identified early because most acutely infected people just show nonspecific flu-like symptoms, or no symptoms at all.

Still, some of the data that supported the 2012 IAS-USA panel's recommendation to treat even acutely infected patients without any symptoms did come from randomized trials. For example, the 115-volunteer randomized Primo-SHM trial showed that, compared with untreated participants, six- or 15-month long ART started within about four weeks after infection lengthened by about 1-2.5 years the time HIV-infected individuals could stay without therapy before they reached a $CD4^+$ cell count of 350 or less (*PLoS Med.* **9**, e1001196, 2012). This means that treating people in the acute phase of HIV infection has an effect on the immune system, which gives them more time to disease progression, says Marlous Grijsen, a physician at the Academic Medical Center at the University of Amsterdam, who was involved in the study. She adds that important early treatment-related changes were $CD4^+$ cell gain and a lower viral setpoint.

Two other randomized trials also studied whether temporary ART that was started within the first six months after infection and stopped between three and 12 months later could delay when volunteers had to restart treatment. One of them, ACTG A5217 (also known as the setpoint study) showed this so clearly that it was stopped prematurely (*J. Infect. Dis.* **205**, 87, 2012). Preliminary results from the other trial, called SPARTAC, also point in the same direction.

Should the SPARTAC trial's final results show similar advantages of early treatment as the Primo and setpoint studies, HIV treatment guidelines should be changed to recommend immediate treatment for acutely infected patients, Grijsen says, provided the advantages and potential disadvantages such as side effects are discussed with each individual.

But others are not so sure. "It's unclear whether there is net benefit or net harm from starting therapy during the acute infection or in the early chronic stage of HIV as opposed to deferral of treatment until the $CD4^+$ cell counts have dropped to lower numbers," says Lundgren, who helped devise the EACS guidelines. "We do not know whether ART used during acute infection or in asymptomatic patients with high $CD4^+$ counts provides net benefit or net harm in terms of morbidity and mortality, compared with a strategy of deferring until the $CD4^+$ cell count has dropped to around 350." He says the EACS guidelines, which currently do not recommend treatment above a $CD4^+$ cell count of 350 unless there are other health issues, are written this way in part because there is no randomized clinical trial that shows that non-fatal disease over-

Continued on page 11

Q&A with NELSON MICHAEL

Andreas von Bubnoff recently caught up with the director of the US Military HIV Research Program to talk about the state of AIDS vaccine research

NELSON MICHAEL

Nelson Michael, M.D., Ph.D., is director of the U.S. Military HIV Research Program (MHRP), one of the main collaborators on RV144, the only clinical trial that has shown efficacy for an HIV vaccine candidate. He played a major role in conducting RV144, in its analysis, and in planning follow up trials. His laboratory collaborates with many other research groups around the world to develop new HIV vaccine candidates.

What's the status of the AIDS vaccine field today?

It's the brightest and most vibrant since the endeavor began nearly two decades ago. The scientific basis for developing vaccines has never been better, in terms of understanding the immune system and protective immune responses, much more consistency across animal models, and the possibility that humanized mice could be effective models for vaccine development. If it was possible to actually do vaccine studies in humanized mice instead of nonhuman primates, it would really electrify the field. Obviously the warp speed with which we've pulled out monoclonal antibodies from HIV-infected individuals that have the properties we want to elicit with a vaccine has been transforming because we are learning so much about how those antibodies need to be generated in terms of the distance they have to travel from the germ line to their fully mutated forms that could be effective. We are beginning to get the first glimmers of understanding that it might be possible to actually use a series of vaccinations to coax those kinds of antibodies out. On the clinical trials side, trials are not proceeding at the pace many would like to see, and I think that's a fair criticism. But I am excited because the major groups that are developing approaches for vaccines for HIV have never worked more collaboratively than these days.

At the recent AIDS vaccine meeting in Boston, the new executive director of the Global HIV Vaccine Enterprise Bill Snow gathered you and other vaccine researchers in what he called a strategic convening session. What happened there?

They brought together a large number of groups that fund HIV vaccine research or execute it, from China, Europe, the US, and Canada. There was such a good positive feeling about how we could work together, could potentially coax new funders into the field, such as approaching the government of China or Thailand to put more of their own government resources into play, for example funding the building of a vaccine plant with the implication that if a trial were successful, outside pharmaceutical companies would come in and help make their vaccine in that country. These are the sort of major players that really haven't been involved before and need to be, because unfortunately, the previous thought process was to look at the existing group of funders and ask for more money. But I think you have got donor fatigue.

In 2003, the article in *Science* that proposed the need for a vaccine enterprise said the vaccine field needed coordination similar to the Human Genome Project. Does the fact that this kind of strategic convening happens now indicate that the coordination isn't there yet?

I think it's a fair criticism. But in the human genome project, we had two groups and very tight control over the activities: A private company, and the US government that funded a number of research groups across the world. For the most part, we knew how to do it, we just knew it was going to take a very long time. With vaccine development, it's very different. You might have hierarchical groups like companies, government organizations and research groups funded by government that all pull in the same direction, but we are still not exactly sure what the roadmap is going to look like, although we have a much better idea than we did a while ago. I think it was only after the STEP study when the field first began to truly collaborate at a level which we have sustained. The unexpected result of STEP forced all of us to quickly work together to figure out what happened. Then RV144

hit, and even though it was good news, it was still surprising, and once again required the field to get together. These things have snowballed now, I think. That's why I am so excited about the field in the past few years because, at every level, you have more collaboration. People aren't being secretive. People are working smarter with less money.

What have we learned from RV144? What's the latest in the analysis of that trial?

The latest is a sieve analysis by Morgane Rolland, a young investigator in our group who published a paper in *Nature* that showed that if you look at the genetic sequences of the viruses that infected vaccinated people in RV144, you saw clear evidence that there was immune pressure on the virus that was induced by the vaccine. That immune pressure was in the second variable loop of Envelope. This is also where a large group of collaborators organized by Jerome Kim in our group and Bart Haynes at Duke described a few months ago where one of the immune correlates was. I think there is more confidence in the initial clinical result of RV144 from the sieve analysis than there was from the correlates analysis. The sieve analysis looked at viruses that came out of the placebo group and the vaccine group, totally randomized, unlike the correlates analysis, where you are looking at vaccinees that became infected versus vaccinees that weren't. The Achilles heel of any correlates study is that, by design, you are looking at just the vaccine arm, whereas sieve analysis is unbiased, in that it compares the control group, the placebo, to the vaccine. Taken together, the initial clinical result, the sieve analysis, and the correlates all pull in the same direction: that a vaccine for HIV is possible and that it worked probably involving an immunological mechanism in the second variable loop.

Will there be follow up trials to RV144, and if so, where?

RV144 was tested in heterosexuals in Thailand at relatively low risk that for the most part only saw a single subtype of virus, B/E. Now we are taking it to South Africa, changing both the prime and the boost from subtype B/E to C. There is tremendous commitment to do a study in South Africa and in Mozambique in southern Africa, where you have a subtype C epidemic. There, largely, the risk exposure is heterosexual, like it was in Thailand, but the incidence is much higher: you are looking at rates of transmission 30-fold higher. Those studies are hopefully going to start around 2015. Multiple companies and groups are involved under the umbrella of the Pox-Protein Public-Private Partnership or P5 led by Gates and NIAID, and MHRP is a part of that group. That is going as fast as we humanly can. The other study in Thailand is using very similar products that were tested in RV144 but, this time, instead of low transmitting heterosexual populations, in very high transmitting populations of MSM [men who have sex with men]. That's where my own group is headed and, at this point, there is not as much enthusiasm as for the study in South Africa. We are trying to get the government of Thailand and other stakeholders in Asia more interested in the process to speed it along and to reduce the risk to the rest of their partners.

What are you hoping for with these follow-up trials?

We are looking for a public health tool to help control the epidemic—for a vaccine that you could actually license in the field. In RV144, the efficacy was 60% in the first year—if that can be sustained with additional boosts or adjuvants, which is going to be tried in South Africa and eventually in Thailand, you would make a substantial impact on the epidemic.

So these follow-up trials could actually end up in a licensed vaccine?

Yes. If a vaccine was tested in Thailand and was shown to be efficacious above 50% in MSM, I think there is a very good chance the Thai government will start negotiating with the drug companies that made those vaccines—in this case Novartis and Sanofi Pasteur—and would potentially license the vaccine for use in very high risk Thais—MSMs or female commercial sex workers. Licensure is up to the national regulatory authorities of any given government and the manufacturers of the drugs: Sanofi, which is involved in making the prime, and for the protein subunit boost, Novartis is taking over protein development previously done by a company called GSID [Global Solutions for Infectious Diseases].

There seem to have been delays in the preparation of the follow-up trials to RV144. Why is that?

The companies weren't ready after RV144, because no one was preparing for a success. That's part of the reason why we have 2012 and still haven't really gone back to expand the studies yet, because it took a long time to sort out how we are going to move forward again. For

STEP, Merck was ready to roll a vaccine out if it had been shown to be efficacious. Not so for RV144, because all the buzz in the field was that that trial was not going to work and a waste of money. Companies were like, well, if the field is not that interested, we can't convince our stakeholders and our board of directors to build a plant ready to roll this vaccine out if it works.

How narrow is the specificity of the vaccines in these RV144 follow-up trials, and how are you going about vaccine development that's applicable to the rest of the world?

The Achilles heel of the current RV144-like approaches is that you are developing vaccines that are really regional, so you don't have a pathway for a universal vaccine. But we are very excited about the work we have been doing with Bette Korber and Dan Barouch and others looking at mosaic inserts. That holds the promise of being able to develop a technique that would allow us to make a universal vaccine. We took the same Ad26/MVA prime-boost approach that we published in *Nature* a few months ago but, instead of SIV inserts, they carry human mosaic inserts, and we showed very good immune responses in monkeys, much like what we saw in nonhuman primates against SIV. And, probably most importantly, we saw protection against a potent SHIV challenge. That's really exciting for us, because now we are seeing evidence that these immune responses actually mean something, so we will start vaccinating humans with these mosaic Ad26 and MVA vectors, approximately in January 2014. The MVAs are made, the Ad26 vectors are in the process of being made. That's not the only approach to make universal vaccines: In Phase 1 studies, we are going to compare those mosaics against multiple single subtype vaccines; for example, a vaccine that contains a subtype B and a B and a C. Gary Nabel is doing this right now in the HVTN 505 study, which is almost fully enrolled. It's a study of MSM and uses DNA as prime and Ad5 as a boost that uses multiple HIV subtypes rolled into one vaccine. It's the only HIV efficacy study that actually is happening right now. We have no results from it yet, but if that one was to work, it would probably work in about 90% of the world's population, so it would also be an approach to make a universal vaccine.

What other promising vaccine approaches do you see?

I am really excited about Louis Picker's work with replicating CMV vectors. Louis has got some tremendous potential I think in these vectors. He vaccinated monkeys with these replicating vectors and then he infected them with SIV and watched as the levels of virus in the blood fell to zero. Then he dissected these animals and looked carefully at their lymph tissues and in some cases now, he is out about a year and a half since these animals were exposed, and he can't find any evidence at all that there is any live virus left. The results are really stunning. Of course, any replicating vector vaccine is potentially more of a risk in people who are immune compromised because of diseases or extremes of age. CMV is a natural pathogen, so you have to really work hard at reducing the risks, but I think Louis is doing a good job at doing that.

These replicating CMV vectors keep producing SIV antigens at a low level and train the cellular immune responses to keep the virus under control?

Right. Louis has seen a phenomenally strong CD8 response. About half of the animals that are vaccinated are protected at some level, at least have a much lower viral load, close to zero, and it looks like some of those animals go on to maybe even cure the infection or to clear it. The obvious question is: How about the other half? So I think Louis is thinking hard about combining his CMV vectors with maybe a protein subunit boost that would produce a lot of antibody, because I think he'd like to be able to have a lot less of the animals infected from the beginning, and Louis can put mosaic inserts into those kinds of vaccines.

So if you take all these promising approaches that are out there, what might a universal HIV vaccine actually look like? Would it be a combination of different approaches?

I would hope it would be something that is simple and deployable that wouldn't require a cold chain. Let's say as an example an Ad26 prime followed by an MVA boost—you could give those two vaccinations within four to six months. If you are going to do a worldwide campaign to wipe out HIV, that's the kind of approach you'd like, other than the RV144 approach—that was six shots given on four occasions over six months, and now it is pretty clear that in the RV144 follow-up trials, we are going to need to give a protein boost at least once a year. That's going to be very difficult to roll out anywhere, let alone in resource-constrained areas.

What other prevention tools are available, and do they make development of a vaccine unnecessary?

There have been great strides in the use of antiretrovirals as preventive measures in microbicides or taken as pills in PrEP or treatment as prevention. I think these are both very powerful tools that have had proof of concepts. But there are lots of challenges to deploy those worldwide—putting a pill in someone's mouth every day is difficult. You can have the most beautiful tool in the world, but if you don't know how to use it or don't choose to use it, it's not going to work. So I think all these are important measures that need to be used together, and I think that in some parts of the world, they may take the edge off the epidemic a bit, but they will never defeat it without a vaccine. I think that's pretty clear from a historical precedent of similar epidemics. I would like nothing better to wipe out HIV infection with treatment —my group is actively engaged in doing it everywhere we work. Our MHRP PEPFAR programs in four countries in Africa give antiretroviral therapy to over 100,000 people. But for every person we put on drugs, there are two others that we cannot get to, so I just don't think it's possible.

Now that Truvada has been approved for PrEP, how could such prevention tools affect how AIDS vaccine trials are conducted?

Will it make some impact? Yes. But experimentally, it's not going to be a problem because as long as people in the placebo and vaccine group equally choose to use or not use those measures, it should statistically not make the study invalid. But it drops the incidence to such a low level that the power of the study is diminished, and I think that's going to be a problem. You have two choices if you have decreasing incidence: You can make the trials bigger—like we do with the RV144 follow-up trials—or you could follow them out for longer. Those are your two choices, and both can add considerable costs.

So what is the future of the vaccine field?

I think it's bright. I think in the next 8-10 years, I am hoping that we will have a public health tool. I think that the pace of success has been rapid. I think it's the most exciting time I have ever seen in the field. ■

Continued from page 7

all (for example, kidney disease), is lower if you treat early than if you treat a little later. Even though today's ARV drugs have far fewer side effects than they used to, he says, "we are running the real risk that there is net harm from using [ART] early in the course of HIV disease."

Even the current drugs have side effects such as bone density loss, and cardiovascular, liver, and kidney damage, adds Günthard. "If this would have the same effect as drinking milk, then there would be no question. But it's not milk." Still, he adds, most observational data do suggest that early treatment is beneficial with respect to non-AIDS defining illnesses such as cancer and cardiovascular disease. So despite the uncertainties, Günthard says he strongly favors early treatment.

But Lundgren and others have initiated a randomized trial called START that, they say, will show if there are any clinical net health benefits—relative to drug side effects and toxicity—from taking the drugs earlier. The 4,000-person trial will, for the first time, examine whether the net health benefits are different if patients start ART above 500 CD4$^+$ T cells or defer treatment until CD4$^+$ counts have dropped to levels below 350. For about five years after patients are enrolled, researchers will monitor patients for AIDS-related and for serious non-AIDS-related events such as heart attacks, stroke, kidney disease, or liver disease. Some of these are known to be side effects of drugs and others are known to be HIV-related.

Not everyone wants to wait that long for answers. Julio Montaner, who runs a program that tests and offers immediate treatment to infected people in British Columbia, says he doesn't need additional data. "[In] every clinical trial, always the higher CD4$^+$ group wins," he says. "How much more evidence do you need before you recommend treatment to all?"

Still, testing is key. Even if very early treatment of acute infection turns out to have benefits for HIV-infected individuals, such as bringing them closer to a cure, these can only be realized if infection is detected early enough. Currently, however, only a small proportion of people are identified during acute infection, says Lichterfeld. "If you were to treat everybody in acute infection, it wouldn't make a huge difference, because the proportion of patients that would be eligible would be very small. So it would not be a major intervention to cure HIV." ■

Shaping the BATTLEFIELD

A host of advances in vaccine design and evaluation promise to transform the campaign against HIV

By Regina McEnery

The cells of the adaptive immune response are, in many ways, the body's elite forces. They may want for face paint and electronic gadgetry, but they patrol their biological perimeter as vigilantly as any contingent of commandos, gathering intelligence and adapting tirelessly to the ever-changing battlefield of the body. When they detect a threat, they respond swiftly, neutralizing turncoat cancer cells, viral saboteurs and invading microbial forces with exquisite precision and breathtaking ferocity.

Yet some enemies prove too canny even for these veterans of microscopic warfare. HIV certainly falls into that category. Not only does it specifically target a key officer in their command structure—the $CD4^+$ T cell—but, thanks to its mutability, does so in ever-changing molecular disguises. When it is targeted by antibodies, the guided missiles of the immune response, it misdirects them with molecular decoys. To further complicate matters, the only neutralizing target open to attack is the Envelope protein, a complex of three identical pairs of proteins—the transmembrane gp41 protein and the extracellular gp120—that is essential to viral invasion of host cells.

But a string of scientific breakthroughs has lately exposed chinks in HIV's formidable defenses. As was evident at the AIDS Vaccine 2012 conference in Boston, Sep. 9-12, researchers are today more confident than ever before that the field of HIV prevention is on the verge of a revolution. Several non-vaccine strategies—including microbicides and an assortment of treatment as prevention approaches— have lately shown promise in large-scale studies, setting the stage for a public health assault that could begin to turn the tide of the HIV pandemic.

The success of these approaches will certainly complicate vaccine development. Yet researchers remain optimistic that steady progress in a number of distinct but convergent strategies for HIV vaccine design and development could soon dramatically alter how we think about the future of the pandemic (see *Q&A with Nelson Michael*, page 5).

If any single aspect of that effort dominated the Boston conference, it was research into broadly neutralizing antibodies (bNAbs) against HIV, scores of which have been cloned and analyzed in recent years. Several laboratories, most notably at the Vaccine Research Center of the National Institute of Allergy and Infectious Diseases and within IAVI's Neutralizing Antibody Consortium (NAC), have in recent years laid the groundwork to devise vaccine immunogens on the basis of information gleaned from their analysis.

The shape of things to come

William Schief, of the IAVI Neutralizing Antibody Center at The Scripps Research Institute in La Jolla, has been developing sophisticated computational methods to reconstruct the epitopes bound by bNAbs. At the Boston conference, he described how he and his colleagues obtained powerful proof of concept for this approach to vaccine design using as a model the respiratory syncytial virus (RSV), the most common cause of lung and airway infections in infants and toddlers. No preventive vaccine against RSV has yet been licensed, but a monoclonal antibody (mAb) called palivuzumab, approved by the US Food and Drug Administration in 1998, can block RSV infection when used to passively immunize premature infants and children with congenital heart disease. Manufac-

tured by Maryland-based MedImmune, it targets an epitope on a protein that is essential to RSV's invasion of host cells. The Schief group selected palivuzumab's epitope for their immunogen design efforts.

Initial efforts to create an immunogen based on an X-ray crystallographic structure of that epitope in complex with motavizumab—an affinity-matured version of palivuzumab—obtained by Peter Kwong's laboratory at the VRC proved disappointing. The first-generation experimental immunogens failed to elicit neutralizing antibodies in mice (*J. Mol. Biol.* **409**, 853, 2011).

That might have been the end of it, but Schief's laboratory didn't want to give up. "We thought we could make better scaffolds," Schief said, referring to the simulated protein structures that are engineered to hold a desired epitope in the appropriate conformation and orientation. Using new code written for Rosetta—a software suite that generates protein structures on the basis of peptide sequences—Schief's lab generated from scratch a variety of possible structures for scaffolds into which the motavizumab epitope could be inserted. The software generated about 100,000 theoretical scaffolds. After filtering the results, the team ordered up eight genes, which were then expressed in *Escherichia coli.*

Six of the eight proteins, Schief said, were "well-behaved." They were very stable and bound very tightly to motavizumab—much more so than structures generated in previous experiments. "The reason we got such tight binding," says Schief, "is that we had frozen the conformation of the epitope." Proof for that came from X-ray crystallographic structures of the unliganded epitope and one bound to motavizumab. They both fit almost perfectly when superimposed over a crystallographic structure of the antibody-bound conformation of the RSV peptide on which they were modeled. This established that the high affinity binding itself was real, not an artifact of aberrant contacts between the antibody and the scaffold.

To test its ability to elicit antibodies, Schief and his colleagues immunized rhesus macaques with various scaffolded epitope constructs and examined their sera for antibodies against RSV. Two assays, including a plaque reduction assay that is considered the gold standard for detecting and measuring the potency of neutralizing antibodies, found that by week 20 monkeys were making antibodies that neutralized a strain of laboratory-generated RSV that is highly resistant to neutralization. Vanderbilt Vaccine Center Director James Crowe, who ran the plaque assay, said the RSV strain neutralized in these assays measures up to strains found in the wild and has been used to challenge healthy adults in clinical trials.

Since macaques cannot be infected with RSV, Schief and his colleagues have no plans to pursue further studies in this animal model. But the preclinical success of the scaffolded epitopes establishes a significant proof-of-concept for an approach that Schief and many other researchers are now applying to design AIDS vaccines. "We were very excited because all of our work on scaffolds [designed on the basis of anti-HIV bNAbs] never produced any neutralizing antibodies," said Schief. "And, you know, everyone rightfully would say, well, maybe scaffolding is never going to work. Maybe there are a lot of inherent problems with designing a little, minimal protein and eliciting antibodies to that that could cross-react to a big, complicated virus. But this experiment shows [our method] actually can work."

A minimalist approach

Structure-based design is not, by a long shot, the only approach to solving the neutralizing antibody problem. Some researchers are trying to strip the sole target on the viral surface—the HIV Envelope protein—down to its bare, immunogenic minimum.

Though essential to viral entry into target cells, the Envelope is a highly variable and structurally dynamic protein. Further, the functional protein offers up many non-neutralizing targets to antibodies, and so misdirects the response. It is covered with a coat of complex sugar chains that are identical to those found on human cells and are therefore ignored by the immune system. Those sugars also obstruct antibody access to potentially neutralizing epitopes on the underlying peptide.

To deal with these difficulties, researchers are using a variety of computational and protein engineering techniques to better focus the immune response on neutralizing targets on the Envelope trimer. Michael Cho, a biomedical science professor at Iowa State University, described in Boston how he and his colleagues stripped gp41 down to its extracellular domain, exposing the conserved membrane-proximal external region (MPER) that lies at the bottom of the intact HIV spike. The engineered protein, said Cho, bound tightly to a trio of bNAbs—2F5, 4E10 and z13e1—that are known to target the MPER domain.

Next, Cho used a histidine tag to link this gp41 fragment to zinc-chitosan, an adjuvant, and immunized rabbits subcutaneously either three times over 16 weeks or four times over 24 weeks. Cho said eight of the nine rabbits mounted bNAb responses. Using the slower immunization regimen, neutralizing activity was observed against 42 viruses that spanned six different clades, including 27 relatively less sensitive Tier 2 viruses. One of the neutralizing epitopes overlapped those for 2F5, 4E10 and z13e1.

Still, the lack of a precise structure for the functional trimer continues to impede its effective use as an immunogen. The closest scientists have come to actually viewing the unliganded form of the functional HIV Envelope glycoprotein has been with cryo-electron microscopy (cryo-EM), including the more refined single-particle cryo-EM (see *IAVI Report* blog, Aug. 20, 2012; *A Slew of Science in Seattle*, *IAVI Report*, Mar.-Apr. 2012). Cryo-EM involves snap-freezing the trimer in liquid nitrogen, taking its image from numerous angles, and then reconciling the images to reconstruct the structure.

A group led by Harvard structural biologist Bing Chen has used single particle cryo-EM to study gp140 trimers, and they plan to submit their structural analysis for publication soon. But Chen cautioned that cryo-EM has its limitations. It can produce inaccurate or misleading structures if the protein preparation being used contains a mixture of monomers, dimers and the like, rather than a homogeneous population of proteins. Chen described the difference between working with these kinds of mixtures as akin to taking 3,000 two-dimensional (2D) snapshots of an animal to reconstruct a three-dimensional (3D) model of the animal, or snapping 1,000 pictures each of a cat, monkey or dog to recreate a 3D model. The latter reconstruction, he noted, would "definitely not look like a dog or a cat or a monkey because the 2D images you used are not from the same object."

To illustrate, Chen showed how the 3D structures of trimers made recently by different laboratories don't all look the same. "At this point, you could not easily disregard one of these reconstructions as the wrong one," he said. "They could be different conformational states. Or some are correct and some are not." But such decisions, Chen pointed out, can have significant practical implications. "An incorrect EM structure of the Envelope trimer will mislead any effort to design or improve immunogens based on that particular structure."

The long path to the bNAb

Even if scientists eventually compute and sculpt their way to the perfect or near-perfect Envelope immunogen, they still need to figure out how to get the body to make bNAbs. Trouble is, they are still trying to work out how exactly these antibodies are made. Studies that applied deep sequencing to trace the genetic pathways of bNAb maturation revealed that those that target the highly conserved CD4 binding site (CD4bs) of HIV, a common bNAb target, tend to share a genetic lineage. They also appear to have been refined over a long period of time, gathering large numbers of mutations in the process of affinity maturation. Many bNAbs also come from B cell lineages known for extremely long heavy chain complementarity determining region 3s (HCDR3s), which is a relatively rare trait.

A robust arsenal of bNAbs has already been isolated from chronically-infected individuals with HIV, and scientists are now studying other producers of these relatively rare antibodies for clues to eliciting bNAbs via vaccination. In one of the largest efforts to date, a study funded by the Center for HIV/AIDS Vaccine Immunology (CHAVI) analyzed the sera of 111 HIV-infected individuals from cohorts recruited by the Center for the AIDS Programme of Research in South Africa (CAPRISA), CHAVI and Amsterdam to measure the prevalence of antibodies that target the CD4bs (*J. Virol.* **86**, 7588, 2012).

Barton Haynes, director of the Duke Human Vaccine Institute and leader of Duke CHAVI-ID—an offshoot of CHAVI—reported that the study found 88% of the samples contained antibodies that bind to the CD4bs. Some 47% contained antibodies to resurfaced stabilized core (RSC) probes, which preferentially bind bNAbs, such as VRC01. The data from each cohort differed some. The analysis found that 2-3 years after infection 31% of the CHAVI subjects and 32% of the CAPRISA subjects had made antibodies similar to those that typically target the CD4bs. But the percentage appears to rise over time: 79% in the Amsterdam cohort—a longer-studied group—were able to make such antibodies five years post-infection. Still, while the antibodies were present, sera from 23 individuals screened against a panel of six heterologous Env pseudoviruses found only modest neutralization potency and breadth, suggesting that the antibodies had not yet had the time to mature fully.

CHAVI-ID and the VRC are now using 454 sequencing to track the genetic lineage and matu-

ration pathways of the antibodies found in the cohorts. They also hope to learn whether there are "blind alleys" in such processes that impede the ultimate generation of bNAbs. The ultimate goal, said Haynes, is to use this information to design immunogens that will guide and accelerate bNAb production.

The long arm of the bNAb

While the practical challenges thrown up by the circuitous pathways of bNAb affinity maturation worries researchers, Crowe thinks it may be possible to design immunogens that elicit high-affinity antibodies without having to go through such a drawn out process. Crowe's work primarily focuses on the long HCDR3s common to HIV-neutralizing antibodies. The bNAbs PG9 and PG16, for example, both have extraordinarily long HCDR3 domains that are essential to epitope binding.

Crowe recently showed by comparing B cells in the peripheral blood at various stages of development that long HCDR3s appear not to be the products of somatic hypermutation (*PLoS One* 7, e36750, 2012). Three different subsets of B cells, including naive B cells and memory B cells, were isolated from the peripheral blood of four healthy HIV-uninfected individuals and four HIV-infected individuals, and their heavy-chain genes sequenced. Crowe and his colleagues reported that the naïve B cell subset encoded a higher proportion of antibodies with long HCDR3s than did affinity-matured B cell groups, suggesting that long HCDR3s are largely generated through recombination and the selection of unusual clones in the early phases of B cell development.

Crowe's lab has also tried to identify antibodies in the B-cell repertoires of healthy donors that might have long HCDR3s that are predictive of the distinctive hammerhead shape of PG9's, which it uses to interact simultaneously with glycans and the protein backbone of Env. To do this, they combed through six million sequences and found about 2,000 HCDR3s that looked to be the right length. Next, they took sequences of around 30 amino acids in length and forced them, *in silico*, to assume the shape of PG9's hammerhead and asked the computer whether it liked what it had.

Only a few sequences were predictive of the hammerhead shape, and the one that Crowe's lab thought would be most predictive ended up not being a good match after all. "But we have another antibody which the computer suggests has the [correct] shape and is able to maintain the shape in the context of a complex. We are predicting that this antibody from a naïve person would interact with HIV."

Crowe said if scientists could figure out how to design an antigen that only binds long HDCR3s, but not the shorter ones, they might get around the problem of bNAb affinity maturation. "People are born with a repertoire that generates these long HCDR3s," said Crowe. "It's just that these [B cell clones] are rare." This, he says, is cause for optimism. "The mystery is trying to stimulate that one in a million repertoire."

Signatures of success

For all the momentum behind neutralizing antibody research, viable vaccine candidates based on bNAbs are not likely to reach clinical testing any time soon. In the meantime, scientists continue to uncover new clues about the only vaccine candidate that has demonstrated protection against HIV—the RV144 regimen, which was found to have 31% efficacy against HIV.

Researchers from multiple laboratories have been analyzing samples collected in the RV144 trial for insights into the underlying mechanisms of that protection. At last year's AIDS Vaccine meeting in Bangkok, investigators shared the first set of results from such analyses, identifying what they called "correlates of risk" associated with the Thai regimen—a vCP1521 canarypox viral vector prime followed by a gp120 B/E AIDSVAX boost (*N. Engl. J. Med.* **366**, 1275, 2012).

Those studies revealed, surprisingly, that one antibody response correlated with a reduced risk of HIV, while another correlated with an increased risk of infection (see *A Bangkok Surprise*, *IAVI Report*, Sep.-Oct. 2011).

Scientists have since turned their attention to the antibody responses that correlated with a reduced risk of infection—namely, immunoglobulin G antibodies that bind to the V1/V2 region of HIV's Envelope protein. Specifically, they have examined whether those vaccine-induced antibody responses selectively blocked certain HIV variants, and what genetic changes allow the virus to elude that targeting. Scientists refer to such escape as a "sieve effect."

Led by researchers at the US Military HIV Research Program (MHRP), a key collaborator in the RV144 trial, the team examined nearly 1,000 HIV genetic sequences from 110 volunteers who became infected over the course of the

[A NEW VIBE IN BOSTON]

The conference in Boston might have been named AIDS Vaccine 2012, but three of its four opening plenary talks had little to do with vaccines. Instead, they covered vaginal microbicides, research into the early initiation of antiretroviral treatment to reduce transmission risk and an overview of what we've learned from trials of pre-exposure prophylaxis. The fourth talk, by Anthony Fauci, the executive director of the US National Institute of Allergy and Infectious Diseases (NIAID), focused on vaccines in the context of these and other preventive interventions.

This underscored a larger point—that the new biomedical prevention strategies to prevent HIV are likely to change both the design and conduct of AIDS vaccine trials. It is likely to make vaccine development more expensive, if the availability of new prevention strategies drives down HIV incidence in cohorts and lengthens the timelines for trials. But it could also set the stage for real world evaluations of comprehensive prevention strategies that have the potential to reverse the tide of the AIDS pandemic.

"The HIV prevention strategy will in fact be a unique paradigm of non-vaccine prevention modalities together with a safe and effective vaccine," said Fauci. The field's advocates have their work cut out for them, though. "We have gotten tired of making our own case," said Bill Snow, executive director of the Global HIV Vaccine Enterprise, an organizer of the conference. "It sounds rote, obligatory and, worst of all, next to impossible. It needn't be that way." It is time, he argued, for the field's advocates to "retune our rationale, refine our refrain. … We can't promise a vaccine by a [certain date] but there are immediate, intermediate questions that will yield to intensive research."

This year's conference was also notable for the prominent role given to young and early career investigators. Two of them, in fact, co-chaired the 2012 conference and about a third of the 46 plenary and symposium speakers were young and early career investigators. If the Boston conference was any indication, they'll have no shortage of subjects on which to build a career. —*RM*

RV144 trial—44 who received the candidate vaccine regimen and 66 who received a placebo. They then examined the viral sequences for evidence that the V2 region plays a major role in the modest protection seen in the trial.

Viruses that bore certain sequences in two stretches in the V2 region of the Envelope gene appeared to be vulnerable to vaccine-induced immune responses; viruses with mutations in those regions of the gene tended to evade such responses. One of the genetic signatures appeared to be associated with an efficacy as high as 78%. "This is an independent assessment that the V2 region is important," said Morgane Rolland, lead author of the study and a scientist at MHRP.

The findings, published in the journal *Nature* the same day they were presented at the Boston conference, buttressed the credibility of the Thai trial results—adding to the molecular evidence that the observed protection was real and not just a statistical anomaly.

On the other hand, they underscored just how difficult it will be to design a broadly effective AIDS vaccine if all it takes to escape protection is a point mutation in a gene that is variable even by the standards of HIV. MHRP Director Nelson Michael acknowledged this fact, but was optimistic that the difficulties can be overcome. "We are making substantive progress in understanding what it will take to develop a more effective HIV vaccine, which will ultimately help us end this pandemic," he said.

In a talk unrelated to the RV144 trial, Rolland reported results from a monkey study that found additional evidence supporting the importance of vaccine-induced responses against the V2 region on the simian immunodeficiency virus (SIV). The findings followed up on an earlier nonhuman primate study in which immunization of rhesus macaques with prime-boost regimens—an adenovirus serotype26 (Ad26) combined with modified vaccinia Ankara (MVA) or with MVA/Ad26—containing *gag*, *pol* and *env* genes from SIVsmE543 resulted in 80% or greater reduction in per-exposure probability of infection against a repeat intra-rectal SIV_{mac251} challenge. Because the challenge virus contained different viral sequences than those in the vaccine candidates, the results were particularly encouraging (*Nature* **482**, 89, 2012).

The follow-up study set out to determine if the observed protection was due to Env-specific antibody responses. To answer this question, 66 sequences from SIV_{mac251} challenge stock and 409 near-full length viral genomes from 13 vaccinated and 13 control monkeys were amplified and evaluated for evidence of a sieve effect. Rolland said that there appeared to be little overall difference in the full-length Env sequences in breakthrough viruses from either group of monkeys. But when they drilled down deeper, they did find evidence of a sieve effect in the Env-V2 segment, suggesting that antibody responses did play a role in the observed reduction in the risk of infection.

Another monkey study, led by Genoveffa Franchini, chief of the animal models and retroviral vaccine section at the US National Cancer Institute, found that a vaccine regimen against SIV analogous to the one used in the RV144 trial induced similar immune responses and outcomes. As was the case in the RV144 trial, the ALVAC-SIV/SIVgp120 prime-boost combination protected a third of the 75 Indian rhesus macaques challenged with a low dose of the highly-pathogenic SIV_{mac251} but did not slow disease progression in animals that were infected. The findings followed an earlier pilot study of 21 monkeys by Franchini's lab that evaluated the same vaccine regimen and reached the same conclusions (see *Tapping the Sanguine Humor*, *IAVI Report*, Mar.-Apr. 2012.)

Stalled trials

Researchers hope to improve upon the results of RV144. But during a satellite session held prior to the opening ceremonies, Jerome Kim, deputy director of science at the MHRP, discussed issues impeding two such planned studies—one involving men who have sex with men (MSM) in Thailand and a second among heterosexual men and women in South Africa.

The Pox Protein Public-Private Partnership, or P5, launched a year ago to boost the vaccine efficacy seen in the RV144 trial to at least 50%, had hoped to launch both studies by late 2012. But a number of setbacks ranging from money to laboratory infrastructure to manufacturing have scuttled P5's initial plans, said Kim. The earliest start date for the southern Africa trial is now pegged at late 2014, and it is unclear when the MSM trial in Thailand will get off the ground. "This is a little depressing," Kim conceded.

The vaccine candidates slated to be tested a Phase IIb trials in Thailand and southern Africa contain immunogens specific to different HIV subtypes. But the candidates in both trials are delivered in a regimen that includes an ALVAC

[HARNESSING INNATE IMMUNITY]

A number of laboratories are developing HIV vaccine candidates that directly activate the innate immune response—especially by engaging dendritic cells, antigen presenting cells that have long been known to play a key role in vaccine-induced immunity.

Preliminary results from a Phase 1 dose-escalation trial of one such vaccine candidate in 45 HIV-uninfected individuals, which was presented at the AIDS Vaccine Conference in Boston by Marina Caskey of Rockefeller University, suggest it is both safe and immunogenic.

The vaccine candidate contains a monoclonal antibody engineered to recognize DEC-205, an endocytic protein found on the surface of dendritic cells that mediates efficient presentation of antigens. The antibody is fused to an HIV clade B p24 Gag protein. Gag p24 was picked as the candidate immunogen because it has many conserved epitopes, and is thus more likely to induce CD8+ T-cell responses against a broad range of viral variants. The vaccine candidate was administered at three different doses, along with an experimental adjuvant called Poly ICLC (Hiltonol) that is designed to stimulate innate immune responses. Volunteers received three subcutaneous vaccinations of either the vaccine candidate or a placebo over 12 weeks and were then monitored for 12 months.

Though the study remains blinded, Gag p24-specific Immunoglobulin G (IgG) antibody responses were detectable in 60% of the volunteers in both low-dose and mid-dose groups at weeks 4,8,12 and 16. Interleukin-2 and tumor necrosis factor-α were the predominant cytokines detected. Antibody responses were durable, with titers unchanged six months after the last immunization. —*RM*

viral vector vaccine candidate as the prime, followed by a gp120 protein boost containing a well characterized adjuvant known as MF59.

Kim said a major challenge in the MSM trial in Thailand has been finding a manufacturer for the proposed gp120 boost. The company that owns the intellectual property rights for the protein boost in the RV144 trial is unable to produce enough for another trial, which means a new manufacturer must be found. Novartis Vaccines and Biologics, in Cambridge, Mass, has the contract to make the protein boost for the southern Africa trial. Kim said Novartis has been asked to make protein for the Phase IIb trial in Thailand as well.

Patchwork protection

Another exciting vaccine strategy involves mosaic antigens—full-length or near-full length proteins that are created by stitching together genetic sequences that represent not only the broadest possible range of HIV variants but that have also been optimized for their potential to induce vigorous and effective immune responses.

Such mosaic immunogens have not yet been tested in clinical trials. But encouraging results from nonhuman primate studies have left researchers hopeful that the approach might be effective in both preventing HIV and in controlling viral replication among those who become infected despite vaccination.

It is, however, unclear how full length mosaic genes stack up against immunogens that encode strings of highly conserved fragments taken from various genes. It is at least possible that unconserved epitopes within full length mosaics will diminish the breadth and strength of responses to their conserved elements—which may be essential to improving the breadth of protection obtained against circulating HIV variants.

To test this notion, a team of researchers led by Dan Barouch at Harvard University compared the breadth and magnitude of the cellular responses induced in rhesus macaques who were immunized with either two or three full-length mosaic Gag, Pol, and Env immunogens, or mosaic immunogens stitched together from conserved regions of those genes. The recently published study used recombinant adenovirus (rAd26) prime in combination with a rAd35 boost to deliver the immunogens (*J. Virol.* **86**, 11434, 2012). "We thought the total magnitude of the cellular immune responses to [conserved elements of] the full-length immunogens would have been diminished, but we actually found the opposite," said Kathryn Stephenson, a scientist in Barouch's lab who presented results of the findings in Boston.

The full-length genes induced, as might be expected, a substantially greater breadth of HIV-specific cellular immune responses. But, contrary to the researchers' expectations, these full length proteins also induced a greater magnitude and breadth of immune responses against conserved epitopes compared to the patchwork immunogens constructed only from conserved regions. The study also found that the breadth of cellular immune responses to the bivalent and trivalent immunizations was comparable. The study suggests not only that full length mosaics might be the better choice, but that simpler, bivalent immunogens work just as well as trivalent ones to elicit a broad response.

In another talk on mosaics, Bette Korber, who heads the HIV Database and Analysis Project at Los Alamos National Laboratory (see *Tracking HIV Evolution*, *IAVI Report*, May-June 2010), reported results from an ongoing immunization study in 36 rhesus macaques that induced remarkable resistance to a low dose, heterologous intrarectal challenge in vaccinated animals. The study is being led by Barouch and Michael of the MHRP, but Korber—whose database furnished the sequences used to build the full-length proteins expressing *gag*, *pol* and *env* genes—presented some of the recent findings during her plenary.

Korber reported that the vaccinated animals received various combinations of Ad26, MVA or Ad35 as either a prime or boost before being challenged with simian-human immunodeficiency virus $(SHIV)_{162p3}$. Korber said the vaccinated animals were able to partially resist heterologous repetitive intrarectal challenge compared to the unvaccinated animals. She said their per-exposure risk turned out to be 80-90% lower than that of the sham-vaccinated controls. "These results are highly significant," said Korber.

She also said the two mosaic inserts evaluated in the different viral vector combinations elicited strong $CD4^+$ and $CD8^+$ T-cell responses to Gag/Pol/Env, and good cross-clade binding antibody responses to Env. This animal study is very much a work in progress, with more data on what may be driving the protection expected soon. Korber did note, however, that an Ad26/MVA viral vector vaccine candidate bearing mosaic immunogens is being manufactured for evaluation in a clinical trial that researchers hope to begin soon. ■

Research BRIEFS

Test and treat may not be best approach in South Africa, according to new study

With the availability of effective antiretroviral (ARV) drugs that have fewer side effects and are easier to take than ever before, calls for the implementation of "test and treat" strategies against the AIDS pandemic are growing in volume and frequency. The idea is to test people regularly and immediately offer antiretroviral therapy (ART) to those who test positive. When World Health Organization (WHO) researchers recently modeled the impact of such an approach on the South African epidemic, they concluded that annual test and treat could eliminate new infections in just 10 years. On the other hand, "universal access"—offering ART to those who are already known to be HIV infected and require treatment—would not only fail to end the epidemic but would cost US$10 billion more over the next 40 years, according to their calculations (*Lancet* **373**, 48, 2009; *PLoS One* **7**, e30216, 2012).

Those conclusions are now being disputed by Sally Blower and Bradley Wagner of the UCLA Center for Biomedical Modeling, who argue on the basis of their own mathematical modeling that universal access is the better strategy for South Africa: Not only would it come close to ending South Africa's epidemic, they say, but would do so at a lower cost than an annual test and treat program (*PLoS One* **7**, e41212, 2012).

One million South Africans currently receive ART, and an additional 1.6 million need it because their CD4$^+$ cell counts are below 350. Blower and Wagner suggest giving ART to the 1.6 million who are known to lack access today, rather than testing all 30 million South Africans and offering ART to all five million people who would be expected to test positive. They agree that test and treat could indeed end the South African epidemic. But they find that universal access would also almost eliminate the epidemic in 40 years, at a cost of $12 billion less than annual test and treat.

Blower says the UCLA model assumes that each year the HIV in 3% of the patients who take ARVs accumulates resistance mutations and that these patients will therefore need to take second-line drugs. As a result, the total number of patients who require such therapies will keep increasing. "We put that into the model because [resistance] is a very serious problem in the US and in Europe," Blower says, adding that in contrast, the WHO model assumed that the percentage of people who require second line drugs remains constant over the years.

The WHO model also assumed that drug treatment makes people 99% less infective, a level Blower and Wagner say is too high because many people don't take their drugs as prescribed or develop resistance mutations. When the UCLA researchers lowered that estimate, their calculations indicated that infected people would have to remain on the drugs for several decades to ensure the epidemic is in fact eliminated.

The UCLA model also assumed that putting people on treatment if they drop below a CD4$^+$ T-cell count of 350 would allow them to live decades longer, much longer than the additional six years the WHO model assumes, Blower says. This results in higher treatment costs and resistance levels for both the test and treat and the universal access strategy. But because test and treat requires putting more people on treatment in general than universal access, these effects are bigger in the test and treat model. This is why that approach ends up being more expensive than universal access in the UCLA model.

Test and treat is "completely unrealistic," Blower says. "If we haven't got the money, talking about these kinds of strategies is nuts. It's pie in the sky both in terms of money and also in terms of actually doing it." Even if the money were available, she argues, test and treat would be logistically difficult in places like South Africa and, given the side effects, not everyone who tests positive would even agree to take the drugs. Providing universal access now, Blower argues, is much better than trying test and treat later. "These are the people who are dying right now," she says.

But Brian Williams, who participated in the WHO modeling, says one flaw of the UCLA model is that the universal access strategy it uses assumes that people should be started on therapy below a certain threshold of CD4$^+$ T-cell counts of 350. CD4$^+$ T-cell counts "are extremely misleading," he says, because in countries like South Africa, CD4$^+$ T-cell counts vary so much that they don't have much to do with how urgently someone needs treatment, or how long someone has been infected.

Williams adds that, contrary to what Blower says, the WHO model assumes that every year, an additional 3% of patients will need to go on second-line drugs. He also does not agree with Blower that the longer life expectancy of people on ART in the UCLA model will necessarily result in an increase in resistance and cost. "If treatment as prevention becomes a reality and there is a market to keep 30 million people on ART, prices will come down," he says. In addition, he says, while the proportion of people on second-line drugs will undoubtedly increase over time, the development of new improved drugs will reduce the problem of resistance.

In the real world, Williams says, drug resistance is not as large a problem as Blower and Wagner assume, referring to studies by Julio Montaner that show that rolling out test and treat in

high risk groups such as intravenous drug users (IDUs) drastically reduced the occurrence of resistance. If monitored carefully, Williams says, resistance is manageable, especially since the combination of drugs used in ART today is much less likely to induce resistance than did previous therapies.

Montaner says he has seen an overall 90% decline in the rate of new cases of resistance in the 13 years he has made ART immediately available for free to everyone infected in British Columbia (*Clin. Infect. Dis.* **50**, 98, 2010), in a program that even involved giving IDUs HIV tests and ART at needle exchange centers. "We actually did it. We didn't model it mathematically," Montaner says. "Test and treat is the way to go. It works, but you have to do it properly: It has to be free, it has to be supported, and it has to be well done." —*Andreas von Bubnoff*

Eliciting elite control—in monkeys

An animal model designed specifically to study elite control of HIV replication has shown that high frequencies of vaccine-induced CD8$^+$ T-cell responses against epitopes on the Vif and Nef proteins of simian immunodeficiency virus (SIV) control viral replication. These responses might be inducible by an appropriate immunization regimen in humans (*Nature* 2012, doi:10.1038/nature11443). The study, led by David Watkins, professor of pathology at the University of Miami Miller School of Medicine, may also provide a way to identify what exactly constitutes an effective T-cell response—not just against SIV, but HIV as well.

The study was conducted in Indian rhesus macaques that express the major histocompatibility complex (MHC) *Mamu*-B*08 allele, whose peptide binding motif is similar to that of human leukocyte antigen (HLA)-B*27, which is enriched among human elite controllers. Eight rhesus macaques were vaccinated with SIVmac239 constructs from three immunodominant T-cell epitopes—Vif RL8, Vif RL9 and Nef RL10—that comprise more than 50% of the CD8$^+$ T-cell responses in SIVmac239-infected *Mamu*-B*08$^+$ elite controllers. The constructs were delivered by a vaccine regimen consisting of a recombinant yellow fever 17D (rYF17D) viral vector prime followed by a recombinant adenovirus serotype 5 (rAd5) viral-vector boost. A control group of eight macaques received an rYF17D/ rAd5 viral-vector prime-boost regimen lacking the genes for the three epitopes of interest.

The vaccine regimen induced robust CD8$^+$ T-cell responses against the epitopes, while the control did not. Both groups of animals developed similar levels of total SIV-specific and CD4$^+$ T-cell responses, as measured by interferon (IFN-*y*) enzyme-linked immunospot (ELISPOT).

Fifteen weeks after the final boost, all 16 animals were challenged intrarectally with a high dose of the highly pathogenic SIVmac239 virus. Four out of the eight animals in the experimental group and six out of eight controls became SIV-infected after the first homologous challenge. The remaining macaques were challenged again three weeks later and all but one became infected. (It took five challenges to infect the lone outlier.)

All eight macaques in the experimental group controlled viral replication during acute infection, while only two in the control group were able to do so. Six of the seven macaques in the experimental group that were infected after one or two challenges became elite controllers, defined in this study as having a set point viral load of less than 1,000 viral RNA copies per ml of blood.

Digging into the weeds a bit, researchers employed peptides and *Mamu*-B*08 tetramers to evaluate potential correlates of viral protection in the monkeys vaccinated with the regimen containing the Vif RL8, Vif RL9 and Nef RL10 epitopes. Peripheral blood mononuclear cell responses against the entire SIV proteome were equivalent in the two groups, but the experimental group with lower viral load had higher insert-specific and proteome-specific CD4$^+$ T-cell responses.

The experimental group also exhibited earlier and higher magnitude Vif RL9- and Nef RL10-specific responses in the peripheral blood, lymph nodes and gut compared to the control group, though responses directed against the Vif RL8 were the same for both groups. The results suggest that the higher frequency of Vif RL9- and Nef RL10-specific responses in blood and tissue were directly responsible for viral control in the experimental group.

The virus did rebound in two of the eight vaccinated animals that received the three targeted epitopes. But sequencing data showed that it occurred following immune escape by all three epitopes, suggesting that the virus cannot replicate in the presence of these CD8$^+$ T-cell responses.

But there are other possible mechanisms by which the macaques might be controlling SIV. Mauricio Martins, a post-doc in Watkins' lab and a co-author of the study, cautioned that there are some notable differences between macaque and human models of elite control. Studies suggest that HIV controllers often have Gag-specific T-cell responses, while SIV-infected macaques do not appear to target Gag epitopes very often. "In fact, the two alleles that have the highest association with elite control [in macaques] do not restrict any immunodominant epitopes in Gag," said Martins. The SIVmac239 strain is also more pathogenic than HIV—the median viral load is 1 million vRNA copies/ml compared to 30,000 vRNA/ml.

Bruce Walker, an expert on elite control and the director of The Ragon Institute of Massachusetts General Hospital, MIT and Harvard considers Watkins' study important for at least two reasons. "It shows that a narrowly-directed cytotoxic T-cell response can be sufficient to control HIV infection, and it shows that manipulation of immunodominance with a vaccine prior to infection can dramatically impact outcome. In other words, it shows proof of principle that very targeted vaccinations can skew the immune response to a better outcome." —*Regina McEnery*

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