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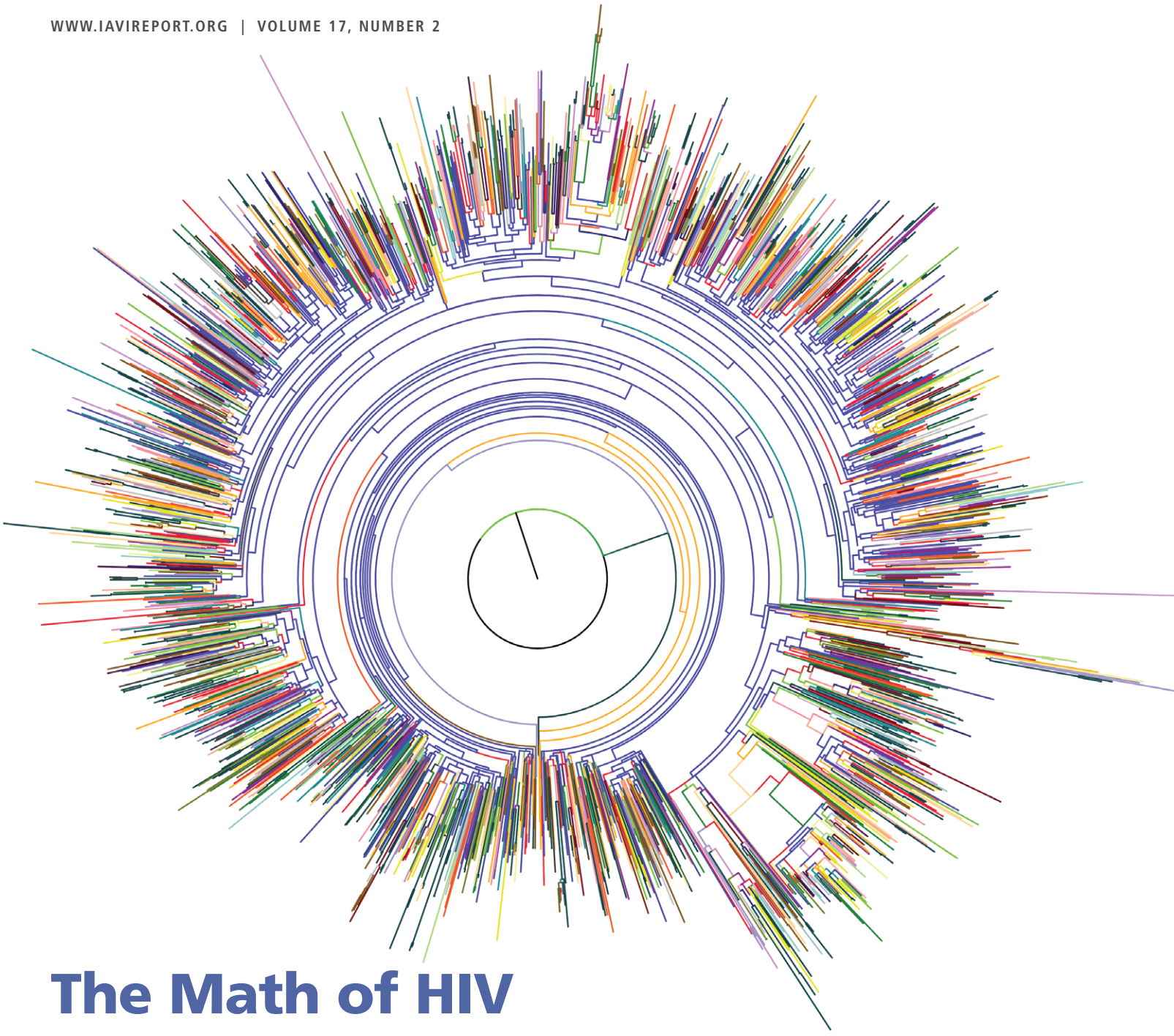


The Math of HIV

Plus:

30 Years of HIV Research

EDITOR'S LETTER

In the three decades since the human immunodeficiency virus was identified as the cause of AIDS, researchers have accumulated an impressive body of knowledge about its transmission and interaction with the immune system. In fact, it's probably safe to say that we know more about HIV than we do about any other viral pathogen. That knowledge has been impressively applied to develop targeted antiretroviral therapies (ART) that have turned HIV infection—once a certain death sentence—into a relatively manageable chronic disease, at least in regions where such therapies are widely available and accessible.

One article in this issue of *IAVI Report* covers a meeting at the Institut Pasteur in Paris, home to one of the two labs that first isolated the virus in 1983, where a number of leading researchers gathered to commemorate 30 Years of HIV Science. Though HIV researchers have plenty to brag about, scientists in attendance focused more on what remains to be done. It is notable that much of the discussion centered on efforts to develop a cure for HIV, an ambition that would have been considered quixotic at best just a few years ago.

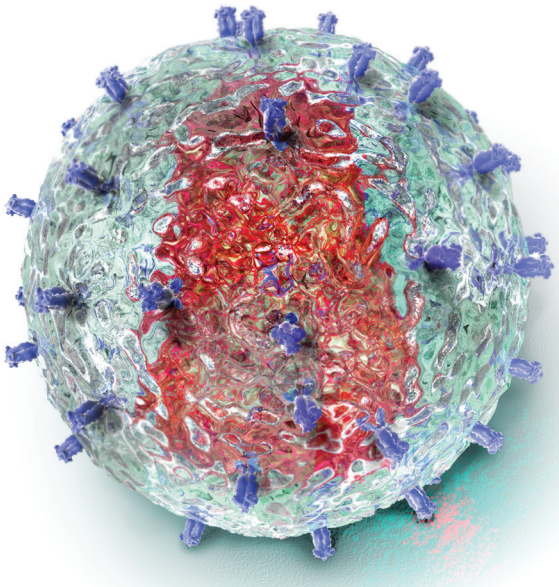
A second major report in this issue comes from a conference held this year in Utrecht, the Netherlands, that brings together experimentalists, mathematical biologists, and theoreticians to explore all things HIV. It was the mathematical modeling of HIV's infective cycle in the body that inspired combination ART, a therapeutic strategy that has transformed the prognosis of HIV infection. All the evidence at Utrecht suggested that such studies still have much to contribute to the prevention and treatment of this pernicious disease. A third report in this issue examines some relatively unusual approaches to designing vaccines to elicit broadly neutralizing antibodies against HIV. Finally, a research report looks at the recent discovery that a candidate vaccine vector, derived from cytomegalovirus, induces highly unusual T-cell responses in monkeys. These might be uniquely harnessed to improve the efficacy of future vaccines.

If all this doesn't sate your appetite for HIV research, we invite you, and like-minded colleagues, to visit IAVIReport.org. Our website is updated every week with blogs and special features covering issues and discoveries of relevance to HIV vaccine development.

We hope, as always, that you will enjoy this issue.

– UNMESH KHER

IN THIS ISSUE



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[ON THE COVER]

Phylogenetic tree showing the relationships between partial *pol* gene sequences (colored radial lines along the perimeter of the circle) taken from 2,084 HIV subtype A infected individuals in the UK. The smaller the branch distance between individual sequences, the fewer transmission events separate infected individuals. The colors of the branches indicate how solidly the data support the branch structure. For example, there is high confidence in the sequence groupings at the tips (yellow) and deep inside the tree (orange), while for the branches in the middle (blue), there is not a lot of confidence about how well the tree reflects reality because the sequences do not contain enough information. For additional details, see page 4.

Image courtesy of Manon Ragonnet-Cronin, University of Edinburgh.

The Math OF HIV

A recent conference in Utrecht was a case study on how much computational biology and mathematical modeling can reveal about HIV and its transmission

By Andreas von Bubnoff

In 1995, a team of researchers including David Ho of the Aaron Diamond AIDS Research Center in New York and Alan Perelson of the Los Alamos National Laboratory published a study that detailed how viral load measurements of a patient treated with a protease inhibitor was used to model viral replication under treatment. Their mathematical model showed that infected cells die after no more than two days, and that most viruses in an infected person therefore most likely come from recently infected cells. This, in turn, suggested that HIV replication could be effectively stopped with a combination of antiretroviral drugs that target different parts of the HIV life cycle (*Nature* **373**, 123, 1995).

The prediction turned out to be correct: Combination antiretroviral therapy (cART) revolutionized the treatment of AIDS, bringing some people back from the very brink of death. The study itself "proved to the field that if you collaborate with mathematicians, you can get new information out of your data," said Rob de Boer of the University of Utrecht and an organizer of the 20th International HIV Dynamics & Evolution conference, held May 8-11 in Utrecht, in the Netherlands. Indeed, he added, it's precisely this kind of work that has created a community of researchers who regularly attend the annual conference.

With its multidisciplinary assemblage of about 140 scientists—including immunologists, mathematical modelers, virologists, and computational biologists—the Utrecht conference offered up a bracing mix of theoretical and empirical discovery in HIV research. The conference also illustrated how mathematical modeling and computational biology can illuminate everything from the evolution of HIV to the networks through which it is transmitted.

Sequences tell tales

Many talks at the conference focused on the analysis of HIV sequences, including the use of those analyses to expose the networks through which HIV is transmitted between individuals. This is possible because many doctors now routinely sequence the *pol* genes of HIV in their infected patients. The gene encodes HIV protease and reverse transcriptase, which are two major targets of ART. Such sequencing alerts physicians to the emergence of resistance mutations and allows them to adjust ART regimens accordingly.

These sequences are particularly easy to collect in the UK, since it is one of two countries (the other is Switzerland) that maintain a national database of the sequences. Currently, the UK database contains about 70,000 partial *pol* sequences, according to Manon Ragonnet-Cronin, who works in Andrew Leigh Brown's group at the University of Edinburgh.

Ragonnet-Cronin presented an analysis of about 2,000 clade A (*see cover image*) and 10,000 clade C *pol* sequences from the UK database, which she conducted to trace the transmission of these viral subtypes. She did this by determining how closely related the sequences are on

an evolutionary tree and how long ago potential transmission events occurred.

Ragonnet-Cronin said that clade A and C infections, once primarily contracted abroad by UK residents, are now predominantly transmitted by heterosexual individuals within the UK and have lately become more common. Her analysis suggests that new infections by these subtypes do not primarily come from highly connected individuals (who transmit HIV to many others), but occur randomly, from well connected as well as less well connected individuals. This would suggest that generalized prevention efforts—which don't target specific groups—are likely to be sufficiently effective in the subtype A- and C-infected heterosexual population.

This is different from a previous analysis of subtype B transmissions, which still dominate in the UK epidemic and occur primarily between men who have sex with men (MSM). In that analysis, Leigh Brown and colleagues showed that MSMs in the UK tend to initially get infected by highly connected individuals. The implication is that, for MSMs, treatment and prevention efforts would be more effective if they were focused on such highly connected people.

Next, Leigh Brown wants to use *pol* sequence analysis to characterize HIV transmission networks in sub-Saharan Africa, to determine which groups of people HIV treatment and prevention efforts should focus on in that part of the world.

US researchers are also using HIV *pol* sequences to analyze HIV transmission. Several US states maintain their own databases. One such state is Michigan, where doctors are required to report sequences to the state, according to Erik Volz from the University of Michigan. Volz presented an analysis of 1,217 partial HIV *pol* sequences collected by the Michigan State Department of Community Health from clade B-infected MSMs.

Volz used sequence similarity and additional data such as incidence, prevalence and stage of infection to show that the epidemic in Michigan is primarily driven by young MSMs who infect other young MSMs. This, he said, belies previous assumptions that the state's epidemic is mostly driven by older MSMs who get infected through transactional sex with younger MSMs.

Volz said this kind of analysis should make it easier to focus treatment and prevention efforts on the right risk groups. Due to a shortage of personnel, he said, it currently takes weeks to interview a newly diagnosed individual and then notify that person's partners. "If you could take [the] personnel and focus them just on the people who are more likely to have transmitted in the recent past, you are more likely to find people with acute infection."

Sanjay Mehta from the University of California in San Diego reported results from a similar study of HIV transmission in the San Diego area. Mehta and his colleagues analyzed over 1,000 mostly partial HIV *pol* sequences collected between 1996 and 2012, mostly from MSMs within weeks to a few months after infection. ZIP codes revealing where the infected individuals resided were available for 565 of those cases. Using this information, Mehta mapped the epicenter of the HIV epidemic in San Diego to the Hillcrest neighborhood and found, to his surprise, a net influx of infections into Hillcrest: More Hillcrest residents had acquired the virus from people outside than *vice versa*.

One explanation, Mehta said, is that more aggressive HIV testing in Hillcrest leads to earlier detection and treatment of HIV infection, which makes infected individuals who live in Hillcrest less likely to transmit HIV to others. If true, this would show that aggressive testing has positive effects and can prevent future infections, Mehta said.

Researchers are also using HIV sequences to study its evolution. Samuel Alizon, a researcher from Montpellier, in France, compared many different sequences to show that HIV evolves more quickly within the same host than between different hosts. Until now, he said, this has only been shown for portions of the *env* gene. But Alizon has found that it is actually the case for the entire HIV genome.

This means that HIV variants that are transmitted to another person aren't the most highly evolved viruses in that person, and that HIV strains that are less adapted to the host have a transmission advantage. In other words, many HIV variants have adapted so much to their host that they lose some of their ability to infect others. The variants that are eventually transmitted, Alizon said, likely come from latently infected $CD4^+$ T cells, where they were probably "stored" from an earlier stage of infection.

Probing the genome

Genome-wide association studies (GWAS) look for links between genetic variations in the host and differences in the way infected individuals respond to the virus. Mary Carrington from the Frederick National Laboratory for Cancer

Research in Frederick, Maryland, uses such analyses to better understand so-called elite controllers—people who naturally keep viral load at undetectable levels for years without treatment. One genetic factor associated with elite control is an allele named B57, which encodes a variant of a human leukocyte antigen (HLA) class I protein that infected cells use to present HIV peptides to $CD8^+$ T cells. It appears that $CD8^+$ T cells kill infected cells more efficiently when they are engaged by the B57 variant.

> We will be able to perform the largest study [of HIV-infected people] ever performed genome-wide. —Paul de Bakker

However, not all infected people who have the B57 allele become elite controllers, suggesting that other genetic factors must be involved in control. To identify them, Carrington and researchers in the lab of David Goldstein at Duke University compared the entire genome sequence of 97 elite controllers who have the B57 allele with the sequence of 90 infected individuals who also have the allele but don't control viral load.

They found that a gene called *KIR3DL1* differed between the two groups. The gene encodes a receptor on the surface of natural killer (NK) cells that recognizes HLA class I molecules on infected cells and then keeps the NK cells from killing the infected cells. The finding suggests that a certain variant of KIR3DL1 synergizes with B57 to control viral load in elite controllers. Carrington said the finding "really solidifies" a previous finding by her group that higher expression of KIR3DL1 synergizes with B57 in the control of viral load (*Nat. Genet.* **39**, 733, 2007).

The mechanism of that control, however, remains unclear. One possibility is that high KIR3DL1 expression on NK cells results in better maintenance of the immune functions of HIV-specific $CD4^+$ helper cells or $CD8^+$ cells. That's because when KIR3DL1 recognizes HLA class I molecules such as B57 on the surface of HIV-specific $CD4^+$ helper cells or $CD8^+$ effector cells, it may keep the NK cells from killing these $CD4^+$ (or $CD8^+$) cells. This would theoretically result in better control of viral load by maintaining the $CD8^+$ related cellular and/or $CD4^+$ related humoral immune response functions.

While this study involved sequencing of the complete genomes of just 187 people, Carrington is also participating in a much larger, international effort to pool genome-wide data of genetic variations from 6,538 HIV-infected people to be used for GWAS analyses. The project, presented by Paul de Bakker from the University Medical Center in Utrecht, does not use complete genome sequences, but "gene chip" data of the most common genetic variations in their genomes.

"We will be able to perform the largest study [of HIV-infected people] ever performed genome-wide," de Bakker said, adding that this will make it possible to identify genetic variants associated with disease progression or viral load control at a larger scale than ever before. It's remarkable, he said, that the HIV research community was able to assemble all their data into one big analysis. "We cannot take that for granted," he said.

Modeling the Envelope

Like many other proteins, the HIV Envelope (Env), which forms the spikes on the surface of the virus, is extensively modified by complex sugar chains. But determining the structure of Env with the attached sugars is difficult to do by X-ray crystallography, which involves growing a protein crystal and then reading how that crystal scatters X-rays.

That's why most available structures of Envelope represent the sugarless proteins. But Natasha Wood, who works in the group of Simon Travers at the South African National Bioinformatics Institute, presented what she said was to her knowledge the first molecular dynamics modeling analysis of a part of HIV Env that included the sugar groups. For her calculations, she used software to attach sugars to known positions of gp120. Using the known crystal structure of gp120, Wood modeled the interactions of all of gp120's 7,500 atoms and roughly 320,000 water molecules surrounding it. It took 64 CPUs 20-24 days to calculate just 30 nanoseconds of the resulting changes in the protein structure.

The analysis focused on the V3 loop, which is thought to determine whether HIV uses the CXCR4 or the CCR5 coreceptor to enter $CD4^+$ T cells, its main target. It showed that with sugar groups attached, the V3 loop of Env is narrower and bent, compared with the V3 of a version of Env that lacks sugar groups. Wood also modeled two proteins, one from a sequence of a virus that has been shown in previous analyses to preferentially use the CXCR4 receptor to infect target cells, with another known to use CCR5 for infection. This showed that the CXCR4 version,

which in this case had one more sugar than the CCR5 version, was a little narrower, in keeping with the overall trend that more sugars lead to a narrower shape.

This suggests that the narrowness of the V3 loop may play an important role in coreceptor tropism, Wood said. But to know for sure, additional proteins known to prefer X4 or R5 would have to be modeled to substantiate the results. Wood also plans to model larger portions of Env, and wants to study how sugar groups affect the interaction between Env and neutralizing antibodies.

Calling all post-treatment controllers

The conference wasn't only about modeling and math. Asier Sáez-Cirión, of the Institut Pasteur in Paris, shared in his keynote address an update of his research on the VISCONTI cohort. This is a group of 14 HIV-infected individuals who started antiretroviral treatment early and, after stopping treatment, turned out to be able to control HIV infection (see *Is it Ever Too Early?, IAVI Report*, Sep.-Oct. 2012). Since he published his results in March, Sáez-Cirión said he has been contacted by researchers from all over the world, and has learned of 20 additional cases of post-treatment controllers. He said that all such cases worldwide will be collected in an international cohort, the formation of which will be announced at the upcoming International AIDS Society conference in Kuala Lumpur.

Sáez-Cirión argued that his and other studies now strongly indicate that HIV-infected people should start treatment as early as possible. That's why he was happy, he said, that the U.S. Preventive Services Task Force recently recommended HIV testing of all people between the ages of 15 and 65, regardless of whether they are at a high risk of HIV infection.

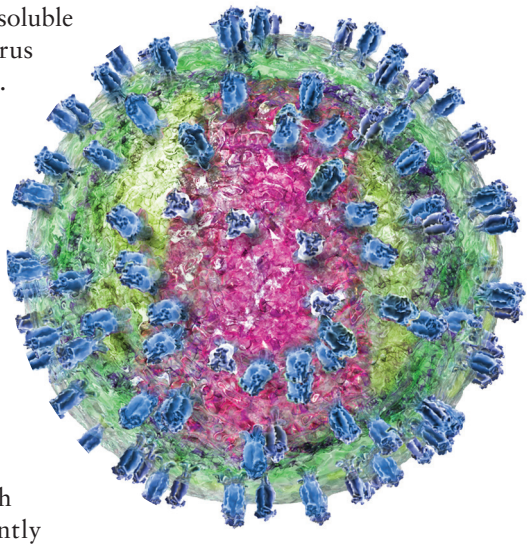
In the discussion after the talk, John Coffin of Tufts University in Boston urged Sáez-Cirión to sequence the HIV variants in the VISCONTI individuals, to determine if there is any ongoing evolution (and therefore low-level replication). That would clarify, Coffin said, if these individuals are more similar to naturally occurring elite controllers (who show ongoing viral evolution) or to people on cART, in whom the virus stops evolving.

New hideouts for HIV

The experimental talks also covered research on the HIV reservoir, thought to reside largely in latently infected, resting memory CD4+ T cells, which divide infrequently. But Ben Berkhout of the University of Amsterdam suggested that multiplying, "activated" CD4+ T cells can also be latently infected, and that contact with dendritic cells (DCs) can rouse the virus in those cells (*PLoS Pathog.* **9**, e1003259, 2013).

This means that in most cases, when the virus infects activated CD4+ T cells, it immediately integrates and becomes latent, which means the cells don't produce the virus. Such latently infected activated CD4+ T cells would thus make up at least part of the reservoir, something that hasn't previously been appreciated, Berkhout said. This, he noted, could also be the way the latent reservoir in resting memory CD4+ T cells is established, since activated CD4+ T cells can turn into resting cells.

To show this, Berkhout and colleagues infected activated CD4+ T cells *in vitro* and blocked new virus infections after four hours. They found that when they added DCs, two to fourfold more CD4+ T cells started to produce virus than in the absence of DCs. This suggests that most of the activated CD4+ T cells had become latently infected with HIV, and that contact with DCs can rouse the latent virus in such cells.



DCs therefore might secrete a soluble factor that can purge the latent virus from activated cells, said Berkhout. "We would like to identify the soluble component secreted by dendritic cells that's doing this," he said. "If you know that component, you may [be able to] produce it as a recombinant protein and use it as a natural purging agent." It could, he said, be used together with other drugs that are currently being tested to purge the reservoir, such as histone deacetylase (HDAC) inhibitors, which induce HIV replication in latently infected resting CD4+ T cells. Because the hypothesized factor would be produced by the body, it would probably have fewer side effects than agents such as HDAC inhibitors.

Coffin also reported evidence for an additional source of the HIV reservoir. He presented a case study of an HIV-infected person who became resistant to his cART regimen after 11 years. The case is unusual because sequencing of his HIV RNA revealed not only drug-resistant HIV, but also a "clonal" population with many identical copies of a wild type, drug-sensitive

HIV variant. While switching the patient's treatment to a new cART regimen reduced the level of the resistant variants, it didn't affect the wild-type variant much, if at all.

Because the patient had oral cancer at the time, it's possible that the wild-type HIV variants came from infected immune cells that were multiplying as part of an immune response to the cancer, Coffin speculated. This would explain the many identical copies of the wild type virus and why cART didn't affect it, he said. Because clonal populations of wild type virus are quite common in patients after about five years of treatment, this could mean that multiplying HIV-infected cells are an additional, underappreciated source of persistent viremia in people treated with cART, Coffin said.

Vaccine updates

While vaccines weren't the main focus of the meeting, there was some discussion of the subject. Hanneke Schuitemaker of the company Crucell gave an overview of current vaccine development efforts at the firm. Crucell has been manufacturing Adenovirus serotype 26 (Ad26) vectors for use in human trials.

As might be expected, one topic that was discussed was the recent termination of the HVTN 505 trial, which was discontinued after it became clear that the DNA/Ad5 prime-boost vaccine regimen it was evaluating did not have any protective effect. The data also revealed a statistically non-significant increase in HIV acquisition among the vaccine recipients compared with placebo recipients. This has raised questions about the future of adenovirus-based vaccination strategies (see *IAVI Report* blog, *HVTN 505: "A hard blow,"* April 26, 2013).

Schuitemaker argued that Ad5 should not get all the blame because it appears that the statistically non-significant increase in infections among vaccine recipients was already apparent after the DNA priming. This, she said, suggests that the DNA priming could at least be part of the reason for the non-significant increase.

Many current HIV vaccine candidates elicit immune responses that the virus easily evades, since the candidates often target HIV proteins—or protein parts—that are not essential to viral survival. As a result, it's easy for the virus to develop escape mutations that don't affect its function, said James Mullins of the University of Washington, who described a vaccine approach that seeks to focus immune responses to essential parts of the virus, so that any escape mutations would harm the virus. This way, Mullins said, the immune system is not distracted into mounting immune responses to inessential parts of the virus.

As immunogens for their vaccine, Mullins and colleagues chose conserved parts of the HIV Gag protein, which mostly form hexamers to create the capsid, a shell that encloses the HIV RNA genome. They found that the parts of Gag that are most important for viral function are the interface portions that connect different hexamers. These are also relatively conserved portions of HIV, and immune responses to parts of the Gag protein have been reported in previous studies to be associated with control of viral load.

Mullins and colleagues made a DNA vaccine that contained seven of these conserved Gag regions. They found that priming with this vaccine followed by a boost with a DNA vaccine containing full length Gag elicited very good $CD4^+$ and $CD8^+$ immune responses in rhesus macaques, as well as vigorous antibody responses. The responses, Mullins said, are initially focused on the conserved Gag elements in the prime, and remain focused on these elements after the boost, which further elevates the responses. This is not the case if the vaccinations are done the other way around, with the full length Gag DNA as the prime, followed by the conserved element DNA as the boost, suggesting, Mullins said, that the prime is the part of the vaccination that's most important to focusing the immune responses.

Because Gag is an internal HIV protein, this Gag-based vaccine approach is unlikely to induce antibodies that prevent infection. But they could reduce viral load, which Mullins wants to check by challenging his vaccinated macaques with SIV. If the vaccine can successfully reduce viral load and focus the immune responses to the conserved elements, then the immunogen "should be the [candidate] Gag component of any vaccine," Mullins said.

It would also be a good idea to try to make a vaccine that contains a version of Envelope that focuses the immune response only on the essential parts of Env. However, he noted, this would be more difficult to develop, because many conserved parts of Env are not essential to its function, making it more difficult to identify the essential parts of Env for use as immunogens.

Perhaps math modelers will present an answer to that problem as well at some future HIV Dynamics & Evolution conference. ■

Mon Dieu! 30 YEARS OF HIV SCIENCE

Leading researchers praise the field's many successes—but remind us the global campaign against HIV is far from over

By Regina McEnery

Now in its fourth decade, the HIV pandemic already ranks among the most devastating in recorded history. And the scientific response has, in some ways, been historic as well. Scientists now know far more about the canny virus that causes AIDS than they do about any other viral pathogen. Even better, their discoveries have led directly to the development of a robust arsenal of antiviral drugs that have transformed both the treatment and the prevention of HIV. So the 500 scientists gathered at the Institut Pasteur in Paris for the 30 Years of HIV Science meeting May 21-23—marking the 30th anniversary of the discovery of HIV at that storied institution—had reason to feel at least a little proud.

But their discussions focused much more on what the next 30 years might look like. After three decades of HIV science, researchers have a profoundly detailed understanding of how HIV hijacks the immune system and exacts its deadly toll, and have figured out how to tame the virus after it has established infection. What they haven't yet worked out is how to stop it before it sets up shop in the body, or how to clear it completely once it has.

Part of the problem is that scientists still don't know how to make a vaccine candidate that elicits broadly neutralizing antibodies (bNAbs), which many researchers believe an AIDS vaccine must induce to prevent infection by the many circulating genetic variants of HIV. And despite growing evidence that HIV might be curable, scientists are just beginning to get a handle on the viral reservoirs that seed lifelong infection.

Longtime director of the US National Institute of Allergy and Infectious Diseases (NIAID) Anthony Fauci praised both the intensive research that has generated powerful antiretroviral therapies, and the global response the drugs have enabled, such as the President's Emergency Plan for AIDS Relief and the Global Fund to Fight AIDS, Tuberculosis and Malaria. "But as we celebrate extraordinary accomplishments," he cautioned, "it is important to keep our eye on the target. Much needs to be done."

A sustained Red Alert

This is indisputably true. Apart from targeting and destroying T cells of the immune system—setting off a destructive cycle that leads to AIDS—HIV appears to induce immune dysfunction in other ways as well. Some scientists believe the virus also overstimulates the immune system, keeping it in such a constant and drawn out state of high alert that it loses its ability to produce immune responses that might control the rapid replication of the virus.

Scientists would like to find new drugs, or perhaps therapeutic vaccine candidates, that dampen or eliminate the effects of such chronic immune activation. So far, however, their efforts have been impeded by an incomplete understanding of the mechanisms of that activation.

Daniel Douek, chief of the Human Immunology Section at NIAID's Vaccine Research Center has been at the forefront of research linking immune activation and the progression of HIV infection. His laboratory has investigated the biological products associated with microbial translocation—the leakage of toxins and other microbial products across the gastrointestinal barrier and into systemic circulation—which they see as a key driver of immune activation and disease progression (see *On the Scientific Trail in Santa Fe*, *IAVI Report*, Jan.-Feb. 2010).

But their attempts at dampening the effects of such activation in rhesus macaques have so far proved disappointing, said Douek. He presented data from a recent study in nonhuman primates designed to assess the impact of blocking a class of secreted immune factors known as type 1 interferons (IFNs) during acute infection by simian immunodeficiency virus (SIV), the monkey version of HIV. While IFNs are known to suppress viral replication, their chronic signaling is also associated with immune activation and disease progression in HIV.

Douek and his collaborators treated six monkeys with an IFN receptor agonist, a drug designed to interfere with the signaling of type 1 IFNs. The researchers then challenged the animals rectally with a pathogenic strain of SIV. They hypothesized that the drug might benefit SIV-infected animals.

In fact, the opposite proved to be the case. Within two weeks, the six animals given the drug had higher SIV RNA levels than a matched group of infected monkeys who had not been given the drug. And it only got worse from there. The treated animals rapidly progressed to AIDS and died within eight months, while the untreated macaques remained alive after 13 months.

Rather than provide a protective effect, Douek said, inhibiting type 1 IFN signaling in acute infection led to long-term loss of viral control and more rapid disease progression in the animals. The big question is, why? Douek's lab is analyzing the data but has no immediate answers. "It's difficult to make sense of this," said Douek. "Clearly, our hypothesis was wrong."

On the frontlines

Several talks in Paris also centered on eliciting immune responses in mucosal tissues, the soft lining of inner body cavities. Vaccines that stimulate such responses could, in theory, be highly effective against HIV, as the sexually transmitted virus establishes a beachhead in mucosal tissues in the early stages of infection.

Ashley Haase, a researcher at the University of Minnesota, is using an unusual monkey model—one vaccinated with a live-attenuated virus (LAV) SIV vaccine candidate—to study mucosal immunity. Haase's group employs the model to look specifically at what happens when antibodies are concentrated on the mucosal frontlines to intercept viruses, but thinks it has applications for any mucosal pathogen. The study also carries special implications for the development of vaccines that target gp41, one of the components of the viral spike—or Envelope protein—that HIV and SIV use to infiltrate cells.

In Haase's study rhesus macaques were immunized intravenously with the LAV SIVmac239Δnef, and challenged vaginally with SIV.

The vaccine regimen itself has a checkered history. In 1992, studies of rhesus macaques suggested that vaccination with a LAV might protect them from SIV. But four years later, high hopes of developing such vaccines against HIV were dashed when the attenuated strain of SIV used in the vaccine regimen mutated into a virulent form, causing disease and death in infant macaques. LAV candidates have since virtually disappeared from the list of strategies favored by AIDS vaccine researchers.

Indeed, Haase is not interested in developing LAVs for vaccines. Rather, his primary interest is in using the macaque model to study mucosal transmission, the most common mode of HIV infection, by sampling tissue immediately after viral challenge. One of the goals of his recent study, he said, was to identify potential correlates of protection—the currently unknown array of immune factors and phenomena that might prevent the establishment of HIV infection.

Haase said when you vaccinate animals with SIVmac239Δnef and then challenge by any route of transmission, there is no significant protection at five weeks. But "wait until 15 weeks or 20 weeks or even 40 weeks" and more than 50% display sterilizing immunity or at least partial protection, he said.

At four and 11 days post-challenge, Haase's team found little or no evidence of a local founder virus population in the cervical opening of vaccinated animals, in contrast to their findings in unvaccinated animals, where there was a heavy concentration of SIV-infected cells. Haase said there was also no recruitment of $CD4^+$ T cells that might have facilitated systemic infection in the vaccinated animals. "Because of the rapidity of this response, we initially thought potentially about an antibody-mediated protective effect," said Haase.

When they looked in the cervicovaginal fluid and cervical tissues of vaccinated animals at five and then at 20 weeks, they noticed a striking, fivefold increase in oligomeric forms of gp41—the transmembrane protein of HIV. "What we think is going on is that the immune system is recognizing trimeric stumps of gp41," said Haase.

Indeed, an early search for clues to what was driving the mucosal immune responses revealed a striking 2.5-fold increase in IgG against gp41 in plasma cells in the submucosa of the cervix. And what initially looked like a wall of plasma cells just underneath the lining of the cervical epithelia turned out to be reserve epithelium express-

ing neonatal Fc receptor—a protein that plays a role in the transfer of IgG antibodies by recycling and stabilizing the antibodies. "During a woman's reproductive life reserve epithelium is thought to provide an anatomical barrier," said Haase. "But here, what we are suggesting is that it provides an immunological barrier as well."

In collaboration with Dennis Burton's lab at The Scripps Research Center, Haase's lab created a soluble form of gp41 minus the membrane proximal external region (MPER) and transmembrane, and tagged it to track down, in the reserve epithelium, specific gp41 trimeric antibodies.

"So for vaccine design, one of the things we think we need to reproduce are antibodies to this trimeric gp41," said Haase. "But [the model] also shows us that we need to understand the rules that regulate how the mucosal epithelium is [established as] the frontline of the immune system and how active a role it plays in shaping the antibody response to concentrate antibodies on the path of virus entry."

While a LAV vaccine candidate probably wouldn't survive regulatory review for human evaluation, Haase said what they're learning from the monkey model could advance the field. "It may be possible to figure out new rules by which the mucosal immune system concentrates its resources where they are needed."

A helping hand

A subset of T cells known as T follicular helper (Tfh) cells plays a central role in boosting the antibody response to pathogens by driving the selection and amplification of B-cell clones to generate increasingly effective antibodies. This process, known as affinity maturation, is of special relevance to the evolution of bNAbs to HIV (see *A Slew of Science in Seattle*, *IAVI Report*, Mar.-Apr. 2012). Recent research has revealed that bNAbs go through an exceptionally lengthy process of affinity maturation that results in the accumulation of extensive somatic mutations that expands their breadth and potency.

Hideki Ueno, an investigator at the Baylor Institute for Immunology Research in Dallas, has been studying Tfh cells for about seven years. His laboratory recently linked a temporary induction of Tfh cells with a protective antibody response seven days after volunteers received a trivalent seasonal flu vaccine (*Sci. Transl. Med.* **5**, 176ra32, 2013).

Administration of the vaccine induced a temporary increase in $CD4^+$ T cells expressing inducible COStimulator (ICOS), which is expressed almost 100% of the time by Tfh cells found in tonsils. Ueno said the induction of ICOS was largely restricted to $CD4^+$ T cells that co-express CXCR3 or CXCR5, one of a handful of sub-populations of Tfh cells identified in recent years. Up to 60% of the ICOS-expressing T cells in his study were specific to influenza antigens, and co-expressed several cytokines, including interleukin 21 (IL-21), which appears to be secreted by Tfh cells to support the survival of germinal B cells.

In vivo studies indicated that an increase in the ICOS-expressing T cells in blood correlated with an increase in pre-existing antibody titers, but not with the induction of primary antibody responses. And *in vitro* studies revealed that purified ICOS-expressing T cells efficiently induced differentiation of memory B cells—though not naïve B cells—into plasma cells that produce influenza-specific antibodies *ex vivo*. All this suggests that the emergence of the ICOS-expressing T cells might serve as an early biomarker for antibody responses, said Ueno.

Although these subsets of Tfh cells may be driving antibody responses in flu vaccinees, Ueno is not sure about their effectiveness in driving B-cell maturation in response to HIV. That's because whether or not this Tfh subset drives potent and long-lasting germinal center responses remains unknown. Further, among blood Tfh cells, the subset expressing CXCR3 is least effective at helping naïve B cells, suggesting their limited capacity to help B-cell responses.

When $CD4^+$ T cells help naïve B cells, they induce the proliferation and differentiation of B cells into antibody-secreting cells, said Ueno. IL-21 is a potent instigator of both events. Although $ICOS^+$ $CXCR3^+$ Tfh cells in blood can secrete IL-21, their capacity to do so is limited, and thus insufficient to help naïve B cells, said Ueno. Similarly, B cells in germinal centers require IL-21 for their survival and proliferation, and Ueno surmises that $CXCR3^+$ Tfh cells are not efficient at helping germinal center B cells, given their limited IL-21-producing capacity. But Ueno said the jury is still out on how to provoke a strong vaccine-induced antibody response in HIV. "There might be other subsets of Tfh that could be more beneficial in HIV," said Ueno.

Chasing a cure

Something approaching a media firestorm erupted when researchers announced at the Conference on Retroviruses and Opportunistic Infections, in March, that a toddler in Mississippi appears to have been functionally cured of HIV infection by the early and aggressive administration of antiretroviral therapy (see *A Toddler Stole the Show*, *IAVI Report*, Spring 2013).

But most of the clinical data in cure research comes from HIV-infected adults, such as the 14 patients in the VISCONTI cohort who started therapy during the acute phase of infection and continued treatment for several years. What makes the VISCONTI cohort unusual, however, is that its members have been able to control their virus for at least a year after interrupting treatment (*PLoS Pathog.* **9**, e1003211, 2013). While three of the individuals in the VISCONTI cohort carry genes for the major histocompatibility class 1 alleles B57 and B26 that are seen in elite controllers, such alleles are not over-represented in the VISCONTI cohort compared to the general French population.

Asier Sáez-Cirión, an assistant professor at the Institut Pasteur who, along with his colleagues, trawled various HIV databases in France to build the cohort, said the common thread among all 14 individuals seems to be a weak viral reservoir that may actually be shrinking (see *Cure Research: Marching on—but over uneven terrain*, *IAVI Report Special Feature*, Sep. 2012).

Sáez-Cirión, who presented an update on the VISCONTI study in Paris, said it could be that early treatment protects the host from chronic immune activation and inflammation associated with more rapid disease progression. Or there could be other host or viral factors at play. In any case, Sáez-Cirión and his colleagues at the Institut Pasteur are now leading an international effort to find individuals like those in the VISCONTI cohort to better characterize the viral reservoirs in such patients. They have already received additional referrals from North and South America, India, and several other European countries. Sáez-Cirión expects the number to grow as researchers and clinicians comb databases.

"What we still don't know is why some individuals can control and why in others this doesn't make a clear impact," Sáez-Cirión noted during his presentation in Paris. "There is something going on. The reservoir is decreasing. We think there is some kind of active control of the reservoir. But we cannot assess this in just 14 patients, so by assembling this larger group of patients, it will help us to make a larger analysis."

Sáez-Cirión said they will be using the larger cohort of patients to better characterize the virus—and in particular the viral reservoir. They also plan to analyze the impact of treatment during primary and chronic infection in the establishment of the viral reservoir.

Steven Deeks, a professor of medicine at University of California-San Francisco, cautioned that given the many caveats associated with cure strategies, a safe, scalable intervention may ultimately prove impossible. In any case, he said, one that works will take decades to develop. He said current antiretroviral therapy is not fully suppressive in many, and perhaps most, people. Nor have any tests yet been developed to measure viral reservoirs in individuals. But he noted that researchers have made considerable progress in unraveling the mechanisms of HIV persistence. "There is," he said, "reason to be optimistic."

One of the HIV cure strategies being studied by Deeks' lab is the use of drugs to reduce the activation and proliferation of T cells, and their expression of CCR5—a surface protein HIV uses to enter and infect T cells. One such drug, sirolimus (a.k.a. Rapamycin), which is used as an immunosuppressant to prevent organ rejection, is being tested in HIV-infected individuals who have also undergone a kidney transplant.

In a study measuring HIV persistence in 91 HIV-infected kidney recipients, Deeks said they found exposure to sirolimus is in some individuals associated with relative reductions in HIV DNA. This suggests it helped shrink the viral reservoir, though Deeks said the reductions were not dramatic. He also said the approach needs to be used with caution. "This is not a benign drug," he said.

Nonetheless, he said plans are being developed for a new study to see if such drugs—known as immune modulators—can be used to block T-cell proliferation. "Bob Gallo would have loved this story," said Deeks, referring to the scientist whose research helped lead to the discovery of CCR5.

In fact, Robert Gallo—who led a National Institutes of Health team that co-discovered HIV 30 years ago—was at the Paris meeting to deliver a much anticipated dinner talk. Part of the buzz stemmed from the other scientist slated to speak, Luc Montagnier, the former Institut Pasteur scientist whose lab also isolated HIV, in 1983. Montagnier bowed out at the last minute. The two labs feuded for a while over who actually discovered the virus first and whose test won the first patent. In 2008, the French team of Montagnier and Françoise Barré-Sinoussi were awarded the Nobel Prize for the discovery of HIV. Gallo was left out.

If there are any lingering bad feelings on Gallo's part, they weren't apparent at the dinner. Gallo talked briefly about his early recollections of the AIDS crisis, when he was drawn into studies of what was then a mysterious and entirely new syndrome. "Scientists got involved quite by chance," he said. "I know I did. When someone challenges you, you take the call." ■

Roads Less *TAKEN*

Most HIV researchers accept that, to be truly effective, an HIV vaccine will have to elicit broadly neutralizing antibodies. What they don't necessarily agree on is how best to elicit that coveted response

By Regina McEnery

Viral vaccines exert their effects mainly by provoking the immune system to make neutralizing antibodies against targeted pathogens (*Cold Spring Harb. Perspect. Med.* **1**, a007278, 2011). But eliciting such responses to HIV is a challenge that has befuddled vaccine designers for decades. This is because the virus has evolved many mechanisms to evade immune targeting. Chief among these is the remarkable mutability, instability, and structural dynamism of the sole antibody target on its surface—the Envelope trimer, or "spike"—which HIV uses to invade the $CD4^+$ T cell.

Yet with the recent discovery of scores of antibodies that appear to neutralize a broad spectrum of HIV's circulating variants, researchers have become increasingly optimistic about designing broadly effective HIV vaccines to elicit similar antibodies.

To that end, they have parsed the structure of broadly neutralizing antibodies (bNAbs) and modeled, in atomic detail, the means by which they bind to their epitopes on the Envelope. The hope, of course, is that this structural information will inform the design of immunogens that might elicit similar responses. And, indeed, researchers are making swift progress toward that goal. They are harnessing structural information to systematically manipulate Envelope proteins for use as immunogens and even reverse-engineer chimeric immunogens that mimic the shape, context, and spatial orientation of epitopes bound by bNAbs (see *Shaping the Battlefield*, *IAVI Report*, Sep.-Oct. 2012). None of these immunogens is anywhere near clinical evaluation. Still, these types of structure-based approaches to vaccine design seem—with fair reason—to be all the rage today.

But some worry that HIV vaccine designers are focusing their efforts on too narrow a range of antibody targets on the Envelope—for instance, the CD4 binding site that is essential to initiating viral entry, or other more accessible epitopes that are known to be targeted by bNAbs. By and large, their objection isn't so much that structure-based approaches are necessarily wrong-headed, or that currently popular vaccine targets on the Envelope are going to prove disappointing. It is more that with the attention showered on reductionist approaches and currently popular epitopes, other vaccine strategies and targets are not getting the attention they deserve.

Going native

James Binley, for one, thinks all the excitement about a narrow set of target structures might be a bit premature. A researcher at the Torrey Pines Institute for Molecular Studies in California, Binley suspects that reverse-engineered immunogens might not be sufficiently immuno-

genic, or even capable of inducing appropriate responses. "The evidence to date suggests that you almost inevitably end up with responses that recognize the immunogen, but that they are off target in terms of being able to recognize the native trimer," he says. "So the [immunogen] is not going to do the job that you want it to do."

Binley and his colleagues are, ultimately, as interested as any other vaccine designer in devising immunogens that accurately capture the structure the Envelope must assume to elicit neutralizing antibody responses. But they approach the problem in a different way. They are trying to construct immunogens that more closely resemble the whole and natural version of the HIV spike that the virus uses to infect cells. Their strategy entails displaying native Envelope trimers on virus-like particles, and embedding the Envelope immunogens in a lipid membrane, as they would be in nature.

This approach has its own challenges. A heterotrimer of the glycoproteins gp120 and gp41, the Envelope's instability and structural dynamism distracts the immune response by offering up a constantly changing cast of structural targets. This is, in fact, what prompted some researchers in the first place to focus on developing engineered immunogens that expose or recreate only small sections of the Envelope as neutralizing epitopes. Others, like Quentin Sattentau of the University of Oxford, have addressed this problem by stabilizing Env, treating it with glutaraldehyde to cross-link certain amino acids and so "fix" the protein in a relatively static structure (see *The Antibody Race*, *IAVI Report*, Spring 2013).

But Binley doesn't do that. His premise is that since the native trimer is responsible for infection and is also the target of neutralizing antibodies, it may be in this form that the Envelope is best used as an immunogen. His laboratory has succeeded in making virus-like particles (VLPs) bearing the native trimer that, in rabbits at least, can induce high-titer neutralizing antibodies. So far, serum from one of the vaccinated rabbits has been found to potently neutralize a primary tier 2 isolate, an achievement that Binley considers a milestone in his career.

Binley ventured down the path to his current vaccine design strategy more than a decade ago, when he was a post doc in John Moore's lab at Weill Cornell Medical College in New York. Binley says it was assumed then that viruses only bore cleaved forms of Envelope on their surfaces. That is, with gp41proteins traversing the viral membrane and the gp120 components arrayed on the outside to form a functional, mushroom-like structure, rather than the uncleaved gp160 monomers. However, his team was accumulating data that suggested otherwise, as were other researchers: the surface of HIV, it turns out, is littered with nonfunctional Envelope proteins. "It was kind of hard getting my brain around breaking that [old] assumption," says Binley. "We had to re-invent the way we think of the virus."

By the time he had started his own lab in 2004, Binley and other researchers were trying to understand why the human immune system couldn't mount a stronger antibody response early on in infection. While it was fairly well established that binding antibodies were a major component of the early response, it was unclear why those antibodies are so ineffectual against the virus. Nor was it obvious how these antibodies differed in their binding from the handful of bNAbs known to science at the time. Complicating the picture was the fact that the non-neutralizing antibody b6 competed with the bNAb b12 for the CD4 binding site of gp120. Yet it did not appear to have any effect on the ability of b12 to neutralize HIV.

It was unclear what this meant. But then Binley's colleague and visiting scientist Penny Moore, who is now a senior scientist at the National Institute of Communicable Diseases in South Africa, led a study that explained the enigma. Her findings, based on studies using VLPs bearing authentic trimers, suggested that non-neutralizing antibodies tend to focus their binding on nonfunctional Env. This implied that nonfunctional forms of Env might serve as decoys, diverting antibody responses away from epitopes on functional Env that are critical to the virus's ability to bind and infiltrate its target cells (*J. Virol.* **80**, 2515, 2006).

The "junk" Env—which includes gp120-depleted gp41 stumps and uncleaved gp160 (*see figure, page 15*)—could thus undermine neutralizing antibody responses against HIV. Binley, meanwhile, suspected that the junk forms of Env were also obscuring the true potential of native trimers as immunogens, and began working with his team to get rid of the stuff on VLPs. He and his colleagues used proteases to strip nonfunctional Env from VLPs, leaving only native trimers intact on the particles—a preparation his team has named "trimer VLPs" (*J. Virol.* **85**, 5825, 2011; *J. Virol.* **86**, 3574, 2012).

"This was our *Eureka* moment," Binley says. "We had tried many other approaches, but there was a firm logic behind this one: that the compact, glycan-encrusted native trimer might be able to survive protease treatments more effectively than the more floppy non-functional forms of Env."

Binley's team then examined the antigenic properties of trimer VLPs. They found that only neutralizing monoclonal antibodies (mAbs) recognized trimer VLPs and that, for the most part, the digests eliminated the binding of all mAbs to the nonfunctional Env consisting of gp160 and gp41 stumps. They also observed that the trimer VLPs retained the ability to infect cells. Their neutralization sensitivity was largely comparable to the undigested, wild-type VLPs, except that they were 100-fold more sensitive to the membrane proximal external region (MPER) bNAbs 4E10 and Z13e1, which suggested an increased exposure of the gp41 base that these antibodies target.

The Binley team later tested these trimer VLPs in rabbits to see what kind of antibody responses they could generate. Binley says serum taken from one rabbit immunized with trimer VLPs showed ID_{50} titers of around 1:1,000 against the JR-FL primary tier 2 HIV-1 strain in the TZM-bl assay. "I think that is pretty tough to get," says Binley, adding that preliminary data shows the specificity of this activity appears to be quaternary and targets a glycan-sensitive region at the base of the V2 loop.

Binley says the vast majority of vaccine studies do not report tier 2 neutralization in the TZM-bl assay, even against the vaccine-matched isolate, which is probably why the more sensitive A3R5 assay is sometimes favored. Binley says they are now working on strategies to elicit this potent activity more consistently and to try to broaden the scope of neutralization.

Rogier Sanders, a University of Amsterdam scientist who has also been trying to generate stable, deglycosylated Env trimers for immunogenicity studies, says removing "junk Env" from Env vaccine preparations is an excellent idea. "It is well-known that most non-functional forms of Env expose highly immunogenic—immunodominant—decoy epitopes that are likely to distract from broad neutralization epitopes," says Sanders, who is also affiliated with Weill Cornell Medical College in New York.

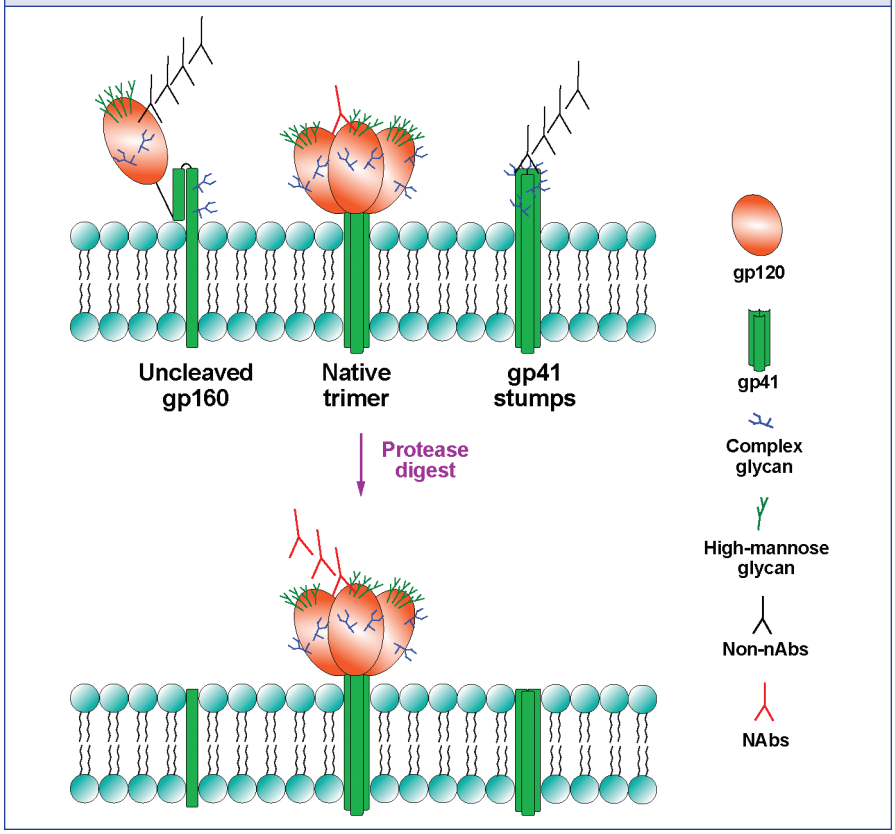
Sanders says the induction of potent neutralization against tier 2 JR-FL is a remarkable achievement. "Sporadic neutralization of autologous tier 2 viruses has been seen before, but not with this potency," says Sanders. "On a cautionary note, the induction of this activity remains sporadic—only one of the rabbits had this neutralization activity. The next bottleneck to tackle is the induction of consistent tier 2 neutralization, even if only autologous."

Binley's group is also collaborating with NIAID's Vaccine Research Center to test the trimer VLPs as baits to isolate new neutralizing mAbs from memory B cells of HIV-infected donors. And they are also in the process of immunizing monkeys with the trimer VLPs to see if neutralizing responses might be easier to induce in this model.

But Binley's approach is not exactly easy to execute. For one thing, the trimers are extremely difficult to make in the laboratory, says Richard Wyatt, director of viral immunology in IAVI's Neutralizing Antibody Center in California, who is collaborating with Binley to test his trimers in nonhuman primates.

HIV-1 virus-like particles (VLPs) and the effect of protease digests

VLP surfaces bear native Env trimers and "junk" Env, consisting of uncleaved gp160 and gp41 stumps. This "junk" Env appears to be immunodominant and promotes non-neutralizing responses, perhaps at the expense of neutralizing responses. Protease treatments selectively remove this "junk" Env but the native trimer survives (lower panel), thanks to its compact nature, coupled with its virtually impenetrable glycan shield. The lack of antigenic interference by "junk" Env in VLP immunogens (depicted in the lower panel) may allow a refocusing of antibodies' attention to the native Env trimer, resulting in more effective neutralizing responses. *Image courtesy of Tommy Tong / Torrey Pines Institute for Molecular Studies*



"You have to use liters [of virus suspensions] to get hundreds of milligrams of the Env on there. It is a lot of work," he says. "And if they work, then you have to figure that it will be a challenge just to get the GMP material to go into a small Phase 1 trial. Then, even if that works, the question becomes, can you scale it up for millions of doses."

But Wyatt says the goal right now should be finding the best immunogen for a vaccine candidate. "If you get something that works," he says, "you may not necessarily be married to that platform. Right now, we don't have a positive control where we elicit broad neutralization. Once we do, there are probably many more ways to make the production of these [proteins] more efficient."

The MPER's domain

If you think trimeric Env is tough, try eliciting viable bNAbs against the MPER region of gp41, a highly conserved site at the stem of the HIV spike. Many HIV vaccine researchers once eyed this region as an attractive target for a universal vaccine because it contains some of the most highly conserved sequences of the HIV genome and plays a crucial role in the fusion of the viral and cellular membranes, a critical step in HIV's invasion of the cell.

But cryo-electron microscopy (cryo-EM) studies to determine the three-dimensional structure of the pre-fusion state of the Env spike suggest this stretch of the viral spike is exposed only transiently during infection (see *IAVI Report* blog, *Structure of pre-fusion state of the HIV Env trimer determined*, Aug. 20, 2012). Further, the MPER domain probably assumes varying conformations, depending on the state of the rest of the envelope. Thus researchers only have a fuzzy notion of the structure of the MPER in the state in which it is available for antibody binding and neutralization.

Still, there's no escaping the fact that a handful of NAbs, including the bNAbs 2F5, 4E10 and 10E8, are known to target epitopes within the MPER. Trouble is, using this region as bait for antibody responses poses some serious problems. Most troublingly, perhaps, antibodies that target the MPER may derive from autoreactive B-cell clones that would be deleted or made tolerant to self-antigen during B-cell maturation. This hypothesis was proposed about a decade ago in a study led by Duke University scientist Barton Haynes, who is today director of one of the two CHAVI-ID virtual centers that are deeply involved in efforts to elicit bNAbs through vaccination. Haynes and his colleagues found that 4E10 and 2F5 cross-react with cardiolipin, a component of the mitochondrial membrane and a target for antibodies implicated in autoimmune disease (*Science* **308**, 1906, 2005).

Other studies have cast some doubt on this finding (*AIDS* **21**, 2131, 2007 and *AIDS* **25**, 1247, 2011), and the recently discovered bNAb 10E8, one of the most potent isolated so far, does not appear to bind self-antigens. There are, however, other problems. Structural studies suggest, for example, that 4E10 and 2F5 epitopes would be relatively inaccessible to most antibodies because they lie at the base of the HIV spike and are partly embedded in the lipid bilayer.

Researchers tried unsuccessfully to produce AIDS vaccine candidates that mimic the MPER domain using synthetic peptides, or by grafting the MPER sequences onto a protein scaffold, or onto proteins displayed on VLPs. Frustrated by failed experiments, many turned their attention to other possible targets on the Envelope, like elements of its glycan shield, its exposed variable loops and, perhaps most avidly, the CD4 binding site.

Yet some recent evidence suggests that strategies to elicit antibodies to the MPER are not necessarily blind alleys on the journey to an AIDS vaccine. Last year, the lab of Mark Connors, chief of the HIV-specific immunity section at NIAID, isolated 10E8, which binds the MPER stalk in an unusual way. Unlike previously identified MPER bNAbs, it doesn't bind phospholipid and doesn't appear to be autoreactive (see *Tapping the Sanguine Humor*, *IAVI Report*, Mar.-Apr. 2012). Its higher potency and breadth of neutralization compared to other MPER bNAbs too has improved the reputation of gp41 as a vaccine target of choice.

Jamie Scott, a molecular immunology professor at Simon Fraser University in Canada, has put together a team to develop DNA, liposome, and VLP vaccine candidates to elicit antibodies similar to 2F5, 4E10 and 10E8, all MPER-targeting bNAbs. "Vaccine research around the MPER has been impeded," says Scott. "People think, 'Oh, those are all auto-reactive antibodies' and so they don't want to work on it. There is huge controversy around this. There are people so tuned into pushing a particular agenda that they may be blind to other possibilities."

Yet researchers have been unable to make immunogens that mimic the structural conformation of the MPER that is targeted by bNAbs.

Scott likened this structure to a "speed bump on the surface of a viral membrane" that literally obstructs antibody targeting. To overcome this impediment, she guesses, you'd need a really flexible paratope—the part of the antibody that recognizes antigen.

Recently, Scott shed some light on an important role the transmembrane domain of gp41 plays in exposing the epitopes of three MPER bNAbs—2F5, 4E10 and Z13e1. The study showed how DNA constructs encoding the MPER, the transmembrane region and 27-amino acid residues of the cytoplasmic tail produced optimal antibody binding to the MPER (*J. Virol.* **86**, 2930, 2012). Importantly, mutants of the 2F5 and 4E10 bNAbs that bind to MPER peptides but do not neutralize the virus also do not bind to the MPER in the context of the cell membrane.

Scott thinks the gp41 transmembrane region helps to fully expose the MPER to neutralization-competent binding by the 4E10 bNAb. She and her colleagues also developed molecular models explaining the difference in exposure of the bNAb epitopes in constructs where the MPER was fused to the transmembrane domain of gp41 and its short cytoplasmic tail, as compared to a longer stretch of the MPER fused to the transmembrane domain of the platelet-derived growth factor receptor.

On the heels of those findings, Scott and her collaborators secured $2.7 million in NIH grants and $330,000 from the Canadian government to develop DNA vaccine candidates targeting the MPER region.

Progress has been uneven. In their initial attempt to design a DNA vaccine candidate that could be expressed on the surface of the plasma membrane, Scott's team realized that not enough of the 4E10 epitope was exposed. "So we turned around and made another vaccine [candidate]," says Scott. The second attempt improved 4E10 epitope exposure, but did not elicit antibodies against the chosen epitope. They are now tweaking the transmembrane region to improve responses in collaboration with William DeGrado's lab at the University of California-San Francisco.

Meanwhile, her attempts to elicit responses to the 2F5 epitope—in collaboration with Shan Lu of the University of Massachusetts—have proved equally vexing. Antibody responses in rabbits immunized with DNA vaccines hit the MPER region targeted by 2F5, but only weakly. To try to improve those responses, Scott and her colleagues decided to pair a DNA prime with a liposome-associated peptide boost developed by José Nieva-Escandón's lab at the University of the Basque Country in Spain. Dr. Nieva-Escandón's lab is developing two liposome-based MPER vaccines: one that covers the 2F5 epitope and another covering the 4E10/10E8 epitopes. They hope that association with the liposome will expose the MPER in a structure that is amenable to binding by 4E10 and 2F5 but not by non-neutralizing antibodies.

Lu is involved in the design of the immunization strategies, and is testing the vaccine candidates in rabbits. While supportive of Scott's hypothesis, he is not quite sure it will ever be possible to get the immune system to produce potent antibodies against the MPER. "We have some [animal] data from previous studies that showed we can generate antibody, at least against [the 2F5 epitope]," says Lu. "Of course, neutralization is not impressive or potent, but at least we hope that we can somehow improve the avidity."

> Vaccine research around the MPER has been impeded. People think, 'Oh, those are all auto-reactive antibodies' and so they don't want to work on it. —Jamie Scott

Scott certainly feels it's worth a try. In her view, too much time and money have been spent assessing the putative autoreactivity of MPER antibodies at the expense of addressing larger questions in AIDS vaccine research. "We need to understand how to drive a B-cell response to make bNAbs," she says. "There are lots of hypotheses about the crucial features of the bNAbs and the B-cells that produce them, and how we can reproduce them with a vaccine. So why are we worrying about deletion of clones? None of the HIV vaccines under development elicits high-titer bNAbs yet—it's not just the MPER vaccines. All the money that has gone into whether [2F5 or 4E10] are autoantibodies could have been used to better understand how bNAb responses are made in the first place."

Other recent findings, meanwhile, bolster the notion that MPER antibodies will be difficult to elicit with a vaccine. A study led by Duke University scientist Garnett Kelsoe identified two self-antigens bound by 2F5 and 4E10 antibodies—human kynureninase (KYNU) and splicing factor 3b subunit 3 (SF3B3). These findings buttress the hypothesis that the autoreactivity of

conserved MPER epitopes represents yet another mechanism HIV has evolved to evade immune attack (*J. Exp. Med.* **210**, 241, 2013). Such mimicry of host antigens, they concluded, is the prime reason for the poor immune responses to the MPER region of Env. Mapping studies of the endogenous antigens targeted by autoreactive antibodies reinforced Kelsoe's conclusion. It turns out that opossums carry a KYNU gene that abolishes 2F5 binding. When immunized with gp140 and then boosted with a peptide immunogen containing the 2F5 and 4E10 linear epitopes, opossums produced high titers of antibody to the 2F5 epitope but little or nothing in response to the 4E10 epitope. For comparison, mice were primed and boosted with the same regimen at two week intervals for 12 weeks. Serum antibody to both the 2F5 and 4E10 epitopes was significantly delayed and, at their peak, antibody responses to both were significantly weaker than those observed in opossums.

Kelsoe and his colleagues argue that this suggests that some humans too might carry KYNU polymorphisms that abolish the 2F5 epitope, suggesting that some people might be able to produce such antibodies. They note, however, that this is for now just a hypothesis.

A recent study led by Haynes might further cheer fans of the MPER. Researchers used a knockin mouse strain expressing 2F5 to show that their immune system eliminates most, though not all, of the B cells that make 2F5 because they are autoreative. But Haynes' group also showed that if apoptosis of bone marrow B cells is circumvented by genetic manipulation, it is possible to rescue B cells bearing self-reactive 2F5 heavy chain/long chain pairs (*J. Immunol.* **187**, 3785, 2011). "If deletion is complete then it would be hopeless to induce these antibodies," says Haynes. "The good news is that the deletion is not complete. A group of anergic B cells can maintain modified heavy and light chains of broadly neutralizing antibodies. The body has not modified them and, with the right immunogen, we can wake them up."

The hunt will not be easy. Just ask Michael Zwick. An associate professor of immunology and microbial science at The Scripps Research Institute (TSRI) in California, Zwick, who worked initially in Scott's lab, actually started out studying the MPER antibodies in Dennis Burton's lab at TSRI. He led the effort years ago to characterize the MPER-binding antibodies 4E10, 2F5 and a third antibody he identified from a phage library (*J. Virol.* **75**, 10892, 2001).

"From there, it became this sort of journey," says Zwick. "The linear peptide epitopes corresponding to these antibodies are relatively straightforward. Antibody binding can be knocked out by a few different residues. We did some early immunizations in mouse models, but the antibody responses were poor. It has been like that for over a decade."

Zwick says some labs, including his own, dug deeper to try and answer some essential questions about the MPER, such as how it becomes exposed on a functional Envelope glycoprotein spike and how much of a window antibodies have to access relevant epitopes on the MPER. But the MPER constructs that they generated induced neutralizing responses that were weak at best.

Such difficulties have sucked the life out of efforts to elicit MPER-specific responses through vaccination, says Zwick. More recently, as potent and broadly effective antibodies that bind choice epitopes have been discovered in scads, interest in the MPER domain has declined precipitously among vaccine designers. Zwick himself has broadened his research efforts to find ways to overcome the instability of the HIV Envelope, the source of much of his current NIH funding. His lab recently combined seven mutations that increased trimer stability, primarily in the gp41 region but also in the V1 region of gp120, to generate a native Env trimer with superior homogeneity and stability (*PLoS Pathog.* **9**, e1003184, 2013).

"One problem with HIV Env," he says, "is if you produce it as a soluble glycoprotein, it does not readily fold in the same way as a native Envelope. It looks different when probing it using non-neutralizing antibodies. Often it doesn't hold together or properly mimic native features of gp41. We thought by selecting mutations it might help get more reliable structures."

Immunizing with VLPs or killed inactivated viruses, says Zwick, induces antibody responses. But often the neutralizing antibody titers are low, despite the generally vigorous antibody responses to Env. "We don't know why this is exactly, and we don't really know how antibody responses are made to Env *in vivo*," says Zwick. "But we are getting much better tools to address this problem, with better mAbs and more ways to study B-cell responses."

Zwick says that he, at least, hasn't abandoned the MPER. "It's too early to tell what might work as a vaccine," says Zwick. "I'm a fan of trying things though."

Even strategies and targets, presumably, that aren't all the rage today. ■

Research BRIEFS

CMV-based vaccine elicits a new kind of T-cell response in macaques

A recent study could change much of our understanding of CD8+ T-cell responses and give vaccine developers new tools to manipulate them.

The current understanding of these responses goes something like this: The body makes millions of CD8+ T cells, each of which expresses a different T-cell receptor. Each of those receptors can recognize a distinct set of peptides that is presented by MHC class I molecules on an infected target cell. When that happens, the CD8+ T cells multiply and develop into effector cells that kill the infected cells or perform other antiviral functions. CD4+ T helper cells, by contrast, are activated by peptides that are presented by MHC class II molecules. Each person has a different set of variants or "alleles" of MHC class I and II molecules. Each can only bind and present certain kinds of peptides. So different people can only make CD8+ T-cell responses to a small subset of the peptide sequences a pathogen contains.

Now a study by Louis Picker of Oregon Health & Science University and colleagues challenges these dogmas. Previously, Picker and his colleagues found that half of rhesus macaques vaccinated with a cytomegalovirus (CMV) derived vector called rhCMV 68-1 that expresses SIV genes could suppress viral load to undetectable levels after repeat rectal challenge (*Nature* **473**, 523, 2011).

In the current study, they report that two thirds of the CMV vaccine-induced CD8+ T-cell responses are not directed at peptides presented by MHC class I, but by MHC class II. These responses are also directed at peptides that are completely different from the "canonical" peptide targets of the CD8+ T-cell responses to SIV infection itself or to other types of vaccines that have been used to deliver SIV genes, including adenovirus vector vaccines. The responses are about three to four times broader than what's usually observed after vaccination with these other vaccines (*Science* 2013, doi: 10.1126/science.1237874).

"It's paradigm breaking," Picker says. "It means that there is a lot more flexibility to [CD8+] T-cell responses than we think. Nothing has been seen before as promiscuous as these responses." Indeed, the responses covered about two thirds of the entire SIV Gag protein, and the responses to some Gag peptides were so common that they were observed in all of about 100 vaccinated monkeys tested.

Apparently, the CMV 68-1 vector itself manipulates the host immune response to behave in such an unusual way: One rhCMV gene inhibits the MHC class I type responses to the "canonical" peptides, while another set of genes enables the broader responses to the unconventional peptides to occur, including the responses to the class II presented peptides. "This is the first time any agent has been found that controls its own recognition by CD8+ T cells," Picker says.

The value of the study, Picker says, lies in the fact that it adds to the vaccine developer's tool box. It might now be possible to make vaccine vectors that modify CD8+ T-cell responses at will.

Both the better breadth and the fact that the peptide targets of the responses differ from the targets to natural SIV infection would make it much harder for the virus to escape from CMV-vaccine-elicited responses, which makes this type of vaccine one of the best current candidates for a therapeutic vaccine for HIV, Picker says.

In a related commentary that appeared in the same issue of *Science*, Nilu Goonetilleke and Andrew McMichael of Oxford University—both not involved in Picker's study—wrote that the findings "could not be more timely," given the recent termination of HVTN 505. That trial used a DNA/Ad5 vaccine regimen, one of several failed HIV candidate regimens that induce the classical narrower MHC class I based CD8+ T-cell responses.

The findings are a "big advance to the field" in terms of the understanding of T-cell immunology in the context of host-pathogen interactions, says Tom Hope of Northwestern University, who was not involved in the study. "It is rather remarkable," he says, "how the CMV vector can change the recognized T-cell epitopes." Still, he adds, the study doesn't clarify which part of these unusual responses helped protect 50% of the vaccinated animals, and why the other half of the animals could not control viral load. "What is not clear is how [the findings relate] to the observed protection," Hope says.

The reason that the study couldn't identify the correlates of the observed viral control in half the animals, Picker says, is that it's difficult to check for immune responses in the tissues relevant for protection such as the mucosa, something Picker and colleagues are doing now. However, Picker adds, all vaccinated animals showed these responses, regardless of whether they were later challenged (and protected) or not, and these were the only immune responses that could be detected in the vaccinated animals. To Picker, this suggests that these responses must be relevant to the observed protection.

So why couldn't some of the animals control the virus, even though all vaccinated animals showed these unusual immune responses? One explanation, says Picker, could be that by chance, the virus might have infected some animals at sites with too few vaccine-induced virus-fighting effector memory CD8+ T cells to contain it.

One concern is that because the induced responses are so broad, the vaccine might induce autoimmune responses. Picker says that that's something to watch for, but adds that so far, there are no signs of autoimmune responses in any of his vaccinated rhesus macaques.

Next, Picker wants to find out how CMV manipulates the CD8+ T-cell response. He has also been working on human CMV vectors that are attenuated to reduce any risks associated with their use in immune-compromised individuals, because the vaccine is based on a replicating vector that permanently expresses its immunogens. "I hope," he says, "to have a phase I clinical trial maybe in the third year from now." —*Andreas von Bubnoff*

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