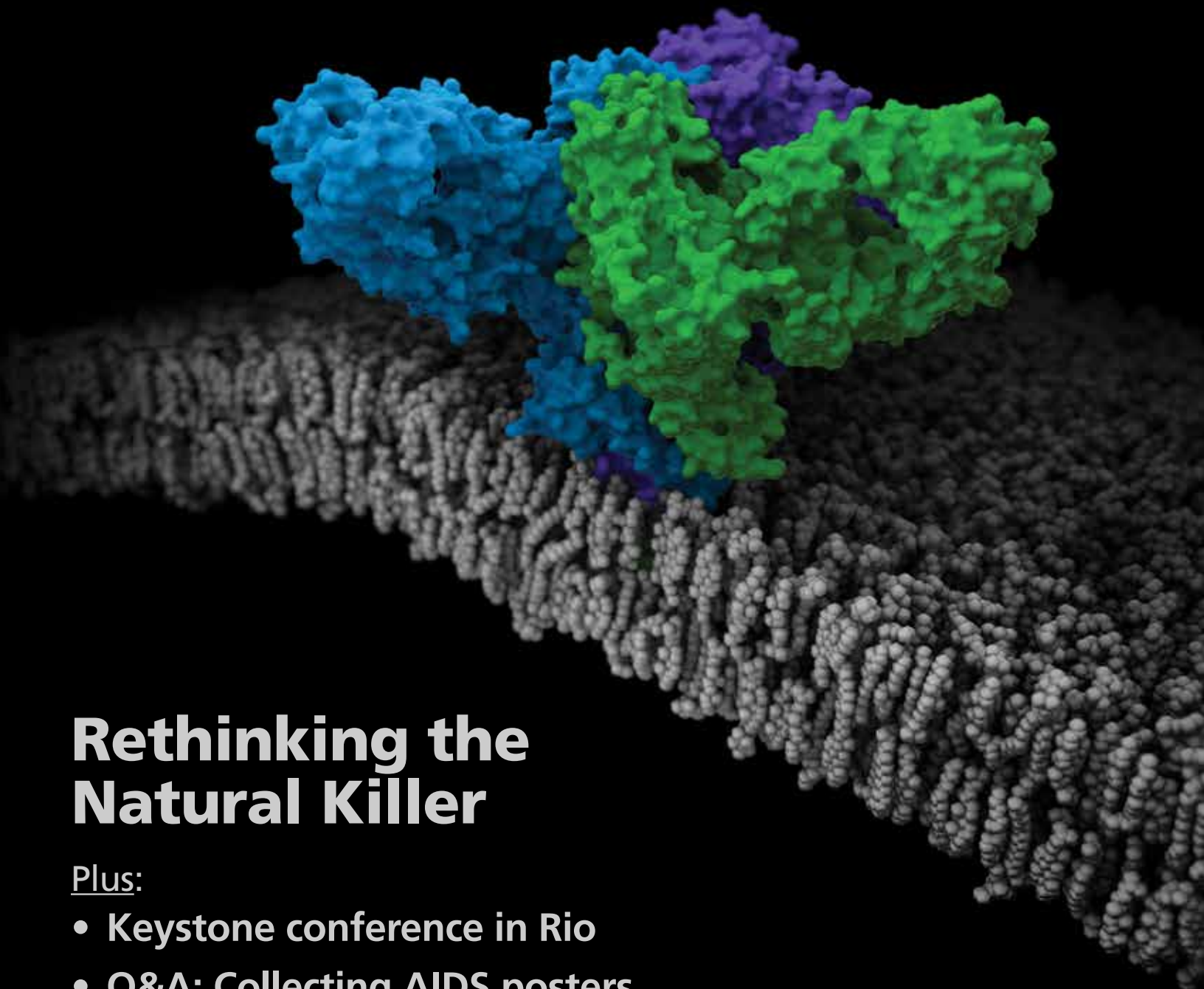


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Rethinking the Natural Killer

Plus:

- Keystone conference in Rio
- Q&A: Collecting AIDS posters from around the world

EDITOR'S LETTER

r

Novelist Isaac Asimov famously observed that “the most exciting phrase to hear in science, the one that heralds new discoveries, is not ‘Eureka!’ but ‘that’s funny...’”

Our lead article bears this out, recounting how an unexpected result from a control experiment ordered up at the last minute led one researcher down a decade-long journey of discovery that adds a new wrinkle to our understanding of the immune system’s organization. Andreas von Bubnoff—who happens to have reported this entire issue—describes in his story a spate of surprising new findings about the natural killer (NK) cell and its apparently remarkable capabilities. Once believed to be a mere foot-soldier of the innate immune response, a lethal foe of diseased and distressed tissue, the NK cell seems to be just as likely an instrument of adaptive immunity, capable of targeting specific antigens and “remembering” what its targets look like. How that remembering occurs is, for now, anybody’s guess.

Our second major story covers the Keystone conference on Advancing Vaccines in the Genomics Era, which was held in Rio de Janeiro. The determination of the structure of a close mimic of the outer face of HIV’s envelope protein continued to make waves at this conference, but a lot of other worthy data was presented there as well—including new findings from systems vaccinology studies.

Next, to change the pace a bit, we’ve included in this issue an interesting Q&A with Edward Atwater, a former professor of medicine at the University of Rochester who collected HIV/AIDS posters from around the world for about a decade and a half until 2005. Be sure to click the links included in our online edition to check out some of the specimens. Finally, our Research Brief this time reports the development of a rapid and cheap new portable system for counting T cells, which could ease the management of HIV infection in developing countries, where HIV has hit hardest.

Finally, a personal note: this is the last issue of *IAVI Report* I’ll be editing, as I’m leaving IAVI. It has been both an education and a pleasure putting out this unique magazine, and I look forward to being a dedicated subscriber. I hope you enjoy this issue, and that you take a moment to visit our Facebook page and hit the “like” button. You can stay in touch with the field that way, or by visiting IAVIReport.org to read our blogs and web specials. I know I certainly will.

On behalf of the *IAVI Report* staff, I wish you a very happy and healthy 2014!

– UNMESH KHER

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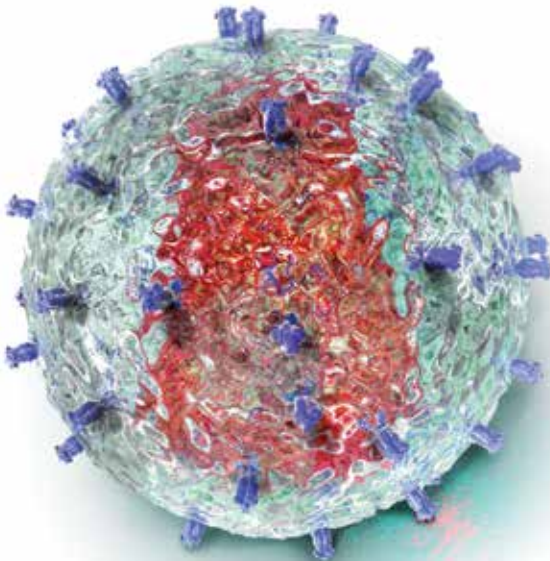
The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996, IAVI works with partners in 25 countries to research, design and develop AIDS vaccine candidates. In addition, IAVI conducts policy analyses and serves as an advocate for the AIDS vaccine field. IAVI supports a comprehensive approach to addressing HIV and AIDS that balances the expansion and strengthening of existing HIV-prevention and treatment programs with targeted investments in the design and development of new tools to prevent HIV. IAVI is dedicated to ensuring that a future AIDS vaccine will be available and accessible to all who need it. IAVI relies on the generous donations from governments, private individuals, corporations and foundations to carry out its mission. For more information, see www.iavi.org.

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[ON THE COVER]

The Envelope (Env) trimer on the HIV surface consists of three identical parts called protomers. The virus uses the Env trimer to enter cells, a process that’s intercepted by broadly neutralizing antibodies (bNAbs) that bind to the trimer. Efforts to elucidate the Env trimer structure have been hampered by its notorious instability, but researchers have recently finally succeeded in making a trimer stable enough for structural analysis. The image shows the 5.8 Ångstrom cryo EM structure of an Env trimer from an HIV strain called BG505, in complex with three molecules of the bNAb PGV04. To create the image, researchers rapidly froze the bNAb-Env trimer complexes, viewed them with an electron microscope from many different angles, and used the images to create the three-dimensional image shown here. Each of the three protomers with bound antibody is shown in a different color (blue; purple; and green, respectively), and the trimer is shown sitting on the lipid membrane of HIV (gray). For the first time, the structure shows the external part of the Env trimer in its entirety, including the relative position of the so-called variable loops 1, 2, and 3. For details, see page 9 of this issue, and *Science* 2013, doi: 10.1126/science.1245627.

Image created by Graham Johnson (UCSF; grahamj.com)

RETHINKING *The Natural Killer*

NK cells have long been considered blunt instruments of the innate immune system. But a growing body of evidence suggests that some NK cells can specifically recognize—and later remember—novel antigens, behaving very much like members of the adaptive immune response.

By Andreas von Bubnoff

Every day, our body makes millions of T or B cells specific to unique molecular signatures, or antigens, most of which will never enter the body. The specificity of each of these cells is determined by a surface receptor—known as the T- or B-cell receptor—that is generated by a constrained but random rearrangement of genes. Though distinct molecules, the receptors borne by healthy B and T cells share the ability to detect antigens that are foreign to the body and so represent a potential threat of infection or disease. When it detects its antigen, the T or B cell becomes activated and proliferates, mounting an immune response against the bearer of that molecule before its progeny die away. A few of its descendents, however, become memory cells, which stay alive for a long time and are rapidly activated if the antigen turns up again.

This “antigen-specific memory” is the hallmark of what immunologists call the “adaptive” immune response. The innate immune system, by contrast, doesn’t have the ability to rearrange antigen receptor genes and therefore cannot respond to or remember specific antigens; its constituent cells—such as granulocytes, patrolling macrophages, and dendritic cells—are less specific in their recognition of quarry, detecting

molecular patterns that are characteristic of certain types of pathogens (viruses, bacteria and so on), rather than specific pathogens.

Natural killer (NK) cells, which destroy stressed or infected cells, have long been thought to belong to this compartment of the immune system. A series of recent discoveries has, however, challenged this long-standing view of their role and capabilities.

Just like B and T cells, it seems, some NK cells can recognize—and later remember—antigens they have never seen before. This has led to a frantic search for some kind of NK receptor analogous to the receptors borne by T and B cells that can somehow change randomly to enable NK cells to specifically recognize random antigens. To further complicate the emerging picture, researchers now have evidence that certain kinds of NK cells can dampen the adaptive T and B cell immune response.

What the new findings could mean for vaccine development is far from clear. But some researchers daringly suggest that NK cells could represent a “third arm” of the adaptive immune system. “People get really excited, [because] this could be huge,” says Stephen Waggoner of Cincinnati Children’s Hospital.

Serendipitous beginnings

It all started with a study of mouse immune responses to haptens in the early 2000s in the lab of Ulrich von Andrian at Harvard Medical School. Haptens are chemicals that can elicit an immune response when they touch the skin. At the time, von Andrian, like everybody else, believed that only T and B cells could possibly mediate hapten-specific memory responses. And, as expected, when he and his colleagues painted the shaved backs of mice with a hapten named dinitrofluorobenzene and, a few weeks later, injected the same hapten into their bladders, the massive inflammatory response involved many T cells.

But as he was preparing to submit the paper, von Andrian asked his postdoc to do just one more experiment: a negative control to show that there is no memory response when B and T cells are absent. To their surprise, the researchers found that the memory response was just as large in mice that lacked those cells, suggesting that perhaps NK cells—one of the remaining immune cell types in these mice—might have been a major driver of the hapten-specific memory response. “So here, suddenly, there was a learned response in an animal that had no T and B cells that led to inflammation,” von Andrian says.

How could this be? At the time, no one had ever reported on NK cells the kind of variable receptors required to elicit an antigen-specific immune and memory response. So von Andrian’s first thought was that the result was a mistake—a mix-up of mice. But a quick check revealed that the mice used in the experiment really had no B and T cells, and when the researchers redid the experiment using similar mice from another source, they got the same results.

Still, von Andrian was skeptical: Perhaps, he thought, this hapten-specific NK memory response was specific to the bladder. After all, it was known that one way to treat bladder cancer is to induce an NK cell mediated immune response to cancer cells by injecting a vaccine. But when the researchers painted one ear of a sensitized B and T cell-free mouse with the hapten (instead of injecting the hapten into the bladder), they got the same result: Only the ear they painted with the hapten was swollen as a result of inflammation. What’s more, this only happened with the same hapten they had used before to paint the shaved back of the mouse a month earlier. “Now you have the hallmark features of

adaptive immunity,” says von Andrian. “It’s a learned response, it’s remembered long-term, and it is antigen specific.”

Around that time, von Andrian heard from other researchers who had made similar observations but dismissed them. “I heard from [a] colleague who had a grad student who showed him these data way before we saw it, and he basically dismissed [it],” he says.

But von Andrian and his team kept digging. They soon found that NK cells were required and sufficient for the hapten-specific memory response, and that only NK cells from the liver of the mice, but not from the spleen, could remember the hapten when they transferred them to another mouse that had no NK cells and had never been exposed to the hapten. This suggests that most memory NK cells reside in the liver, von Andrian says.

To add a final layer of confidence in these results, von Andrian let his postdoc repeat the key experiments in a blinded fashion. “It always came out right,” he remembers. “So I was very confident at that time that what was observed was a real reproducible effect that was not tainted by observer bias.”

Still, he had sleepless nights because he felt that this was “just too heretical to be true.” And he wasn’t the only one with doubts: The manuscript spent eight months at one of the major scientific journals, went through three revisions, and an editor told him at one point that a reviewer had called the study “the cold fusion of immunology.” Around the same time, the third postdoc who had been working on the project quit. “[She] actually decided that [she] did not want to stay in science,” he says. “At the time, this project was so frustrating that no one in my lab would touch it with a ten-foot pole.”

That’s when von Andrian had enough. On a Friday night, he sent the study to *Nature Immunology*, which accepted it the following Monday without further review (von Andrian had informed them about the many previous revisions and changes to the manuscript; *Nat. Immunol.* 7, 507, 2006).

The paper made quite a splash. “That was a big discovery,” says Marcus Altfeld, an NK cell expert who still has a lab at the Ragon Institute in Boston but is currently moving to the Heinrich Pette Institute in Hamburg. Still, researchers were scratching their heads: How could NK cells respond to anti-

gens they had never seen before without the kinds of variable receptors that T and B cells are known to have? The only types of NK cell receptors known at the time were hardwired (encoded in the germline, so that all NK cells had the same receptors), and they did not appear to undergo routine genetic rearrangement. For example, some NK cells in certain strains of mice that are naturally resistant to cytomegalovirus (CMV) infection carry a CMV-specific receptor called Ly49H on their surface, probably because CMV has been infecting mice for millions of years, which is long enough for NK cells to evolve the receptor.

Challenging [old] ideas is just tough. We are probably more likely to discard [a] result rather than thinking that it might be an initial indication that there is a completely different kind of arm of the immune system. —Marcus Altfeld

But when von Andrian reported in 2010 that mice can also show NK memory responses to antigens of HIV (*Nat. Immunol.* 11, 1127, 2010), a hardwired receptor seemed like an unlikely explanation because HIV is a relatively new virus and doesn't infect mice. "I think the data really challenged that idea of a germline-encoded receptor that has emerged through evolution," Altfeld says. They suggest, he adds, that NK cells might indeed have a receptor that can randomly rearrange to bind novel antigens.

Meanwhile, several other groups have also detected NK cell-based hapten responses, von Andrian says, and Norman Letvin at Harvard Medical School and his colleagues reported that an NK cell-based memory response can protect mice from vaccinia virus infection (*PLoS Pathog.* 7, e1002141, 2011). Even NK cells that carry hardwired receptors like the CMV receptor Ly49H have been shown to form long-lived memory NK cells: When UCSF's Lewis Lanier, who initially found the Ly49H receptor, transferred CMV-specific NK cells from one mouse to another mouse that lacked these CMV-specific NK cells, he and his colleagues found that the transferred cells rendered the mice protected from CMV infection, persisted for at least six months, and enabled the mice to respond to a secondary CMV challenge with a stronger immune response (*Nature* 457, 557, 2009).

Is NK cell memory hardwired?

While there's strong evidence that the kind of antigen-specific NK memory von Andrian found is real, nobody knows how it is formed. "Could it be," von Andrian asks, "that there is perhaps a [genetic] locus in NK cells where some kind of gene becomes edited or rearranged, where you generate [receptors with] highly diverse, more or less randomly formed binding specificities, similar to the T- and B-cell receptor locus?"

Such a scenario isn't completely improbable. After all, von Andrian argues, adaptive immune memory cells that undergo genetic rearrangements of receptor genes that differ from B- and T-cell receptors have been found in primitive vertebrates called agnathans (jawless fish), which include hagfish and sea lampreys. Their immune memory is formed by so-called "variable lymphocyte receptors."

Another possibility is that NK cell memory could be encoded not by rearrangements of the genes, but by so-called "epigenetic changes," such as chemical modifications of the genetic material itself to silence various genes in response to environmental stimuli.

von Andrian reasoned that if the memory can be transferred between different cells together with the genetic material, it's likely based on genetic rearrangements, since any epigenetic changes would be erased after transferring genetic material. And indeed, he has done preliminary experiments in mice that suggest that the NK cell memory doesn't get erased and has even started to identify the part of the genome that might carry the NK cell memory. "The most likely, simplest explanation," von Andrian says, "is that there is actually a gene, a locus where there is some modification at the DNA level that's heritable that determines NK cell specificity for a large diversity of different antigens."

Despite these results, not everyone is convinced that an epigenetically encoded NK cell memory can be excluded. For one, DNA sequence itself has recently been found to encode instructions as to how to change DNA epigenetically, says Waggoner. In addition, Lanier says that when he and others compared the expression of all of the about 20,000 genes of NK cells before and after immunization of mice, they didn't find any obvious candidate receptor genes that were expressed differently. That's why Lanier is currently checking whether changed methylation patterns of genes could explain NK cell memory.

But, in general, the field seems to be slowly accepting that antigen-specific NK cell memory exists, and is eagerly waiting for the identification of the elusive NK cell receptors. “I think there is [now] much greater acceptance,” says von Andrian. “As of two months ago, for the first time after having worked on this for ten years, I was able to get NIH grant support.”

Altfeld agrees. “I think [most] people are now—even in the NK cell field—accepting it,” he says. “That was different two years ago. But the big question now is what kind of receptor is mediating that. The Holy Grail right now is trying to identify that receptor that mediates this antigen specificity.”

If von Andrian is right, he might have identified a third arm of the adaptive immune system—which was actually the title of one of his rejected grants. There are fewer NK cells than B and T cells, he admits, but on a per-cell basis, NK cells seem more powerful, because fewer virus-specific NK cells than T or B cells need to be transferred to naive mice to render them protected from influenza infection.

While most of the evidence for NK cell memory comes from mice, there are already hints that it might also exist in nonhuman primates and perhaps even humans. Keith Reeves of Beth Israel Deaconess Medical Center, in collaboration with von Andrian and Dan Barouch of Beth Israel Deaconess Medical Center and the Ragon Institute, studied NK cells from rhesus macaques that had been vaccinated years ago, and found that liver and spleen NK cells from the animals could kill dendritic cells from the same animal *in vitro*, but only if these dendritic cells had been exposed to the same antigen that was in the vaccine the animal had seen five years ago. This shows that in rhesus macaques, the NK cell-based antigen-specific memory can last as long as five years.

One hint that even humans might have such responses is von Andrian’s observation of NK cell memory responses to viruses or haptens in humanized mice, which carry human NK cells.

But if the macaque experiments are any indication, studying human NK cell responses could be a challenge. That’s because the NK memory cells in rhesus macaques reside mostly in the spleen and liver, from where they probably migrate to the sites of infection. If this is also the case in humans, Altfeld says, it will be difficult to get samples of memory NK cells, unless research-

ers can identify an NK memory cell marker that can be used to identify such cells in blood, where they are much rarer. The ideal marker, of course, would be the elusive antigen-specific memory NK cell receptor itself, Altfeld says. “The critical step really is to identify that receptor that mediates antigen specificity,” he says. “Once that receptor is identified, then I think we can really characterize these NK cells in more detail.”

NK cells as wet blankets

If the recent findings on NK memory cells aren’t complicating things enough, researchers now also have evidence that NK cells can have a dampening effect on the B- and T-cell response.

While it’s been known for decades that NK cells can regulate adaptive immunity, the first evidence that this regulation is of vital importance for control of virus infection came from a 2011 study by Waggoner and his colleagues. Waggoner says that one motivation for the study was when he heard of a somewhat paradoxical finding by Mary Carrington and her colleagues a few years ago: An NK cell receptor that *inhibits* NK cell activity was associated with *better* control of HIV and hepatitis C infection, which would imply a stronger immune response.

So how could lower NK cell activity be associated with better immune responses? Most people, Waggoner says, tried to reconcile the result with their assumption that NK cells primarily kill virus-infected cells, and thought that perhaps only NK cells with a functioning inhibitory receptor (or “brakes”) get the license to kill: “They are only putting gas in the cars that have a brake pedal,” he says.

But, Waggoner wondered, what if NK cells dampen the adaptive T-cell immune response? This would suggest a different explanation of how an NK receptor that inhibits NK cell activity could lead to better control of HIV and hepatitis C infection: A better adaptive immune response. To see if this could be the case, he infected mice with a strain of lymphocytic choriomeningitis virus (LCMV) called clone 13, since clone 13-infected cells are less likely to be killed by NK cells. That’s important because he didn’t want to have any NK cell effects on the adaptive immune response obscured by the fact that NK cells kill infected cells.

It was known at the time that mice can not only clear low doses of LCMV, but also survive high doses, because the virus overwhelms the

immune system so much that it stops fighting the virus, which then simply persists because the animal's immune response is weakened. In addition to low and high doses, Waggoner and colleagues also tried a medium dose in their study. They found that the medium dose could kill the mice because there was enough virus to keep the immune system fighting, but not enough to overwhelm it sufficiently to give up the fight. The continuing immune response eventually killed the mice (*Nature* 481, 394, 2011).

But when the researchers depleted the NK cells in the mice, they saw the opposite. The mice survived the medium dose, but died at the high dose. Waggoner's explanation was that at both doses, NK cells kill activated CD4⁺ T cells, dampening the adaptive immune response, and that removing NK cells strengthens the CD4⁺ and CD8⁺ T-cell response. At the medium dose, this helps the mice clear the virus. At the high dose, though, the stronger CD4⁺ response so boosts the response that it continues fighting the virus, which eventually kills the mice.

The NK cell-mediated killing of CD4⁺ T cells also has a dampening effect on the antibody response, Waggoner and colleagues found. Without NK cells, mice that are acutely infected with LCMV not only have stronger CD4⁺ and CD8⁺ memory T-cell responses, but also develop LCMV-specific neutralizing antibodies sooner.

Not much is known about similar effects of NK cells in humans, but researchers have recently reported that in HIV elite controllers, there is an inverse relationship between the strength of NK cell responses and virus-specific CD8⁺ T-cell responses, Waggoner says (*AIDS* 26, 1869, 2012). To find out more, Altfeld is checking the effects of NK cell activity on adaptive immune responses in people acutely infected with HIV, and is studying whether depleting NK cells in humanized mice can improve the adaptive immune response to vaccination.

Implications for vaccine design

Waggoner's results would suggest that a vaccine that can inhibit NK cells that kill activated CD4⁺ T cells should do a better job at inducing broadly neutralizing antibodies. On the other hand, vaccines that can induce memory NK cells that can kill infected cells could also be quite advantageous, because such NK cells seem to respond more quickly than T cells when it comes to migrating to the places where they are needed,

such as mucosal sites, says Altfeld. "NK cells have this very quick response time before they kill, much faster than T cells," he says. What's more, von Andrian says, NK cells can't be infected by HIV, whereas vaccines that induce T cells might make HIV infection worse by creating additional HIV target cells.

Still, the opposing effects NK cells can have on the immune response—improving the immune response by killing infected cells, but dampening the adaptive immune response by killing activated CD4⁺ T cells—represent quite a challenge for vaccine developers. For example, a vaccine or drug that inhibits NK cells that kill activated CD4⁺ T cells would have to do so without also inhibiting NK cells that can kill infected cells. Alternatively, a vaccine that induces NK cells that kill infected cells would have to do so without also inducing NK cells that inhibit adaptive immune responses. Selectively modulating just one of these opposing NK cell functions will require that researchers completely understand both, Waggoner says. Importantly, von Andrian notes, these opposing effects of NK cells on the immune response could very well reflect the effects of different types of NK cells.

While it's unclear how to develop a vaccine that induces effective NK cell responses, researchers are starting to study the effects of existing vaccines on NK cells. Altfeld and colleagues reported a few years ago that flu vaccination results in changes in the types of NK cells that persist for at least 150 days after vaccination. Altfeld and Reeves plan to study NK cell responses in volunteers of vaccine trials, and Lanier is part of the human immunology project consortium where researchers use microarray or similar analyses to check responses to vaccines such as yellow fever, flu, and, in Lanier's lab, to *varicella zoster* (chickenpox) vaccine in a more comprehensive way than ever before.

As is often the case in scientific discovery, the recent findings have raised more questions than they have answered. But it is equally clear that scientists, like all people, are often reluctant to shed their prejudices; were that not the case, the NK cell's versatility might have been discovered earlier. "Challenging [old] ideas is just tough," Altfeld says. "We are probably more likely to discard [a] result rather than thinking that it might be an initial indication that there is a completely different kind of arm of the immune system." ■

KEYSTONE IN RIO: Breakthroughs, Predictions, and Surprises

Recent breakthroughs stole the show, but the meeting also highlighted the growing importance of systems biology and the use of sequence information in immunology and vaccinology

By Andreas von Bubnoff

The timing couldn't have been better. On the very first day of the Keystone meeting on Advancing Vaccines in the Genomics Era from Oct. 31 to Nov. 4 in Rio de Janeiro, *Science* published a series of papers matching the splashiest talks delivered at the conference: The structure of a near-native version of the HIV Envelope (Env) trimer (see cover image), and the proof that, in principle, a structure targeted by a potent neutralizing antibody can be used as a starting point to design a vaccine immunogen, at least for respiratory syncytial virus (RSV). But while these findings clearly stole the show—Novartis researcher and conference co-organizer Rino Rappuoli called them “breakthroughs,” a word rarely heard from researchers—attendees also learned about an impressive array of advances in the application of systems biology to vaccine design, and how genomic sequences can be used to explore differences in immune responses to both vaccines and infections.

The fine structure of the HIV trimer

The notorious instability of the HIV Envelope trimer has long hindered efforts to obtain its molecular structure at a truly useful resolution. But an effort led by John Moore at Weill Cornell Medical College in New York has over the years identified ways to stabilize the protein without disrupting it too much, and settled on one called BG505 SOSIP.664 for further analysis. It comes from an HIV clade A founder virus (i.e. the one that initially caused infection) isolated from an infant in Kenya.

Ian Wilson and Andrew Ward from The Scripps

Research Institute presented the results of the structural analysis of the BG505 trimer by X-ray crystallography and cryo-electron microscopy (EM). Wilson, who presented the X-ray crystallography work (*Science* 2013, doi: 10.1126/science.1245625), said he and his colleagues tried to grow crystals of BG505 bound to many different broadly neutralizing antibodies (bNAbs), and found that the best crystals formed when BG505 was bound to the bNAb PGT122. Ward and colleagues used cryo-EM to determine the structure of the same BG505 trimer bound to a different bNAb, PGV04 (*Science* 2013, doi: 10.1126/science.1245627; see cover image).

The structures obtained through X-ray and cryo-EM confirm and complement each other, said Ward, who presented the cryo-EM work. For the X-ray structure analysis, researchers had to shave most sugars off the trimer to crystallize it, while cryo-EM allowed them to leave on all of the sugars.

At a resolution of 4.7 Ångstroms, the X-ray structure doesn't quite reach atomic-level resolution, but Ward is confident that it's accurate because it agrees with the 5.8 Ångstrom cryo-EM structure, and with the previously determined structure of monomers of gp120, as the external part of Env is called. “This is really it,” he said, adding that one challenge now is to further improve the structures to get closer to atomic-level resolution. Ward also wants to make the cryo-EM structure even more similar to the native trimer by adding back the so-called membrane proximal external region, which the researchers removed to make the trimers more

soluble and to prevent them from clumping together.

For the first time, the BG505 structures show the external part of the Env trimer in its entirety, including the relative position of the variable loops 1, 2, and 3. “The biggest thing we learned was that the epitopes are a lot more complicated than previously thought,” Ward said, referring to the target sites of bNAbs. This complex environment restricts the angle an antibody can come in to bind. For example, he said, “you have to come in and navigate this very straight path in order to get to the CD4 binding site.”

The studies have also taught researchers how to make other stable trimers. “[BG505] is a stable trimer, and we know that it doesn’t fall apart, so it can be used as an immunogen,” Wilson said, adding that the next goal is to make similar trimers from other HIV clades. “It’s really the start of a new generation of immunogens and vaccines that weren’t previously accessible,” Ward added.

While the BG505 structures are consistent with each other and with a cryo EM structure published on Oct. 23 by Sriram Subramaniam and colleagues (*Nat. Struct. Mol. Biol.* 20, 1352, 2013), they differ from a previous structure published by Joseph Sodroski and colleagues (*Proc. Natl. Acad. Sci.* 110, 12438, 2013). Sodroski’s structure, which has been the focus of controversy fueled by critical comments published by leading researchers, therefore remains an outlier and requires further validation of accuracy, Ward said.

A future RSV vaccine?

Peter Kwong of the Vaccine Research Center at the U.S. National Institute of Allergy and Infectious Diseases reported the design of a candidate vaccine against RSV. The strategy used could also be important for HIV vaccine design, as it shows that, in principle, structures targeted by potent neutralizing antibodies can be used as potential immunogens.

Earlier this year, Kwong and colleagues determined the crystal structure of D25, a potent neutralizing antibody to the prefusion form of RSV F, the protein the virus uses to enter its target cells.

In the new study, they used their knowledge of this structure to develop a stabilized version of the prefusion RSV F protein without the D25 antibody bound to it and used this stabilized protein to immunize mice and monkeys (*Science* 342, 592, 2013). They found that it induces neutralizing antibodies at levels many times higher than the titers needed for protection, and at least 10 times higher than the titers induced by a post-fusion form of the RSV F protein, which is currently being developed as a vaccine candidate. Next, Kwong and colleagues

plan clinical trials with the new RSV F immunogen.

Conference co-organizer Bali Pulendran from Emory University was clearly impressed with the RSV results. “This is one of the first examples of an approach where you can go from a structure through rational design to construct an immunogen that’s highly immunogenic,” he said.

Kwong and other researchers are trying a similar approach—using a structure bound by a highly potent antibody as an immunogen—to design an HIV vaccine. D25-like antibody responses to RSV seem very common in people, so one important message of the RSV study for the HIV field, Kwong said, is that it’s important to choose an antibody as a starting point for such efforts that’s not just broadly neutralizing and potent, but also commonly made by people. “It’s very, very important to look at what humans make naturally at high titer. If you want a vaccine that everyone could make, see what people make,” he said, adding that he is currently performing detailed analyses to find the most common bNAbs in HIV-infected people.

Good inflammation, bad inflammation

Several talks shed an interesting light on the complicated role of inflammation in vaccine responses. Glenda Canderan from Rafick Sékaly’s lab at the Vaccine and Gene Therapy Institute of Florida reported that a signature of changes in inflammation-related genes in elderly people before vaccination corresponds with lower responses to flu vaccination. This suggests that age-related systemic inflammation is one reason why vaccines have less of an effect in elderly people.

But inflammation is only bad for vaccine responses if it gets out of control, Sékaly said. Normally, the body tries to keep inflammation in check by expressing certain genes (such as one called *SOCS1*) that dial it down. But in people who show lower vaccine responses to yellow fever, Sékaly didn’t find such genes significantly switched on, suggesting that only inflammation that’s unregulated lowers vaccine responses. This, he said, should be taken into account for vaccination strategies in developing countries, where inflammatory responses to other pathogens could lower the response to vaccination. Canderan has preliminary results that suggest that unregulated inflammation might also explain lower responses of elderly people to influenza vaccination. Next, Canderan and Sékaly want to test if reducing inflammation before vaccination can improve responses to yellow fever and hepatitis B vaccines.

Bonnie B. Blomberg of the University of Miami School of Medicine also looked at inflammation in

elderly people. She found that elderly people make more of the inflammation-mediating cytokine TNF- α , which correlates with a lower response to flu vaccination. What's more, elderly people have fewer B cells that have switched to IgG, the antibody type that's most relevant for an immune response. Blomberg's findings suggest this is probably because stimulated B cells of elderly people make less AID, an enzyme involved in antibody maturation and switching to IgG. She found that the level of AID (and TNF- α), as well as the number of switched memory B cells can predict vaccine responsiveness in elderly and young people.

Higher preexisting inflammation in elderly people might therefore lower their B-cell responses to vaccines. One possible remedy might be to restore AID function, Blomberg said. She cautioned that this might have side effects, though this could be circumvented by giving treatment only at the time of vaccination. Another strategy could be to reduce inflammation. One way of doing that without inducing side effects, she said, is surprisingly simple, at least in theory: meditation, stress reduction, a healthy diet, exercise, or social support.

Blomberg noted that there are two types of inflammation. The bad sort is chronic inflammation, which leaves the immune system less room to respond to a vaccine with a proper, acute inflammatory innate immune response. In chronic inflammation, Blomberg said, B cells can become refractory to an antigenic stimulus. "You want to make an inflammatory response when you get the bug [or vaccine], but not before," she said.

One question researchers are still grappling with is why in the Step trial, people with preexisting immune responses to Ad5, the vector used in that trial, showed increased HIV infection risk. Alan Aderem of the non-profit Seattle Biomedical Research Institute reported that one day after vaccination with the Step trial vaccine MRKAd5, people with preexisting immunity to Ad5 activated fewer inflammation-related genes, suggesting that insufficient activation of appropriate "danger signals" by the vaccine may have something to do with the increased HIV infection risk in this population.

Certain genetic defects can also modulate inflammation in people, which can lead to serious problems, according to findings by Dan Kastner of the National Human Genome Research Institute of the NIH. Kastner and his colleagues have established a cohort of about 1,900 patients with so-called autoinflammatory diseases whose causes are unknown in most of the patients.

The cohort includes two young children with an extremely rare combination of symptoms: recurrent high fever and strokes. To find the reason for this, Kastner and colleagues sequenced the part of the genomes of the two children and their parents that codes for proteins. Assuming that both copies of a gene need to be mutated to cause the disease, they found that both children shared a mutation in two copies of only one gene, which could explain the disease.

The defect was in a gene called *CECR1*, which encodes a protein called ADA2 that is thought to be important for the development of certain white blood cells. To test whether mutations in *CECR1* can cause strokes, Kastner and his colleagues inhibited the expression of a *CECR1*-related gene in zebrafish embryos. And indeed, they found that the fish developed strokes within 48 hours and had impaired white blood cell development, effects that could be reversed by introducing the normal version of the human gene into the embryos.

They then used these clues to take a closer look at the defects in the children and found that they, too, had impaired development of a certain type of white blood cell that keeps inflammation in check, in addition to inflammation around blood vessels and problems with the integrity of their blood vessel walls. The combination of these problems could explain the development of fever and strokes in these children.

These insights have already led to the identification of additional cases, including one child whom Kastner and his colleagues first learned about on the MSNBC web site. The North Carolina boy suffered from unexplained strokes and his doctor, a pediatric neurologist, told the host of the "Today" show that he had never seen a case like this before. "He was sure that there was no other case like it in the United States," Kastner said. "Well, we had four [cases], so we called him up and sure enough, that child also had two mutations in this gene."

The findings may eventually lead to treatments of the condition, Kastner said, for example by replacing the missing protein by gene therapy. They could also shed light on the cause of adult strokes, because Kastner and colleagues found one mutated copy of the *CECR1* gene in two adult brothers, both of whom had strokes in their 70s.

HLA alleles and immune responses

Small variations in a tiny part of the genome that encodes HLA molecules can have dramatic consequences for the quality and vigor of responses to both vaccines and infections. That's because

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immune cells use HLA molecules on their surface to present tiny parts of immunogens called peptides to activate CD4⁺ and CD8⁺ cells, which play key roles in both the antibody and cellular immune response.

Differences in the exact sequence of these HLA molecules matter, because they determine which parts of an immunogen HLA molecules present to CD4⁺ and CD8⁺ T cells. Researchers have found that such differences can result in different responses to vaccination. Tomer Hertz of the Fred Hutchinson Cancer Research Center in Seattle reported evidence suggesting that certain HLA alleles made some vaccine recipients in the RV144 trial—the only one to have detected any measure of vaccine-induced protection from HIV—more likely to be protected from HIV. He told attendees that HIV virions that could infect RV144 volunteers despite vaccination were more likely to have mutations in peptides that are preferentially presented by an HLA class I variant called A*02, which presents HIV peptides to activate CD8⁺ T cells. This suggests that vaccinees with HLA A*02 were more likely to be protected in RV144.

And indeed, Hertz found that the vaccine efficacy was 54% in RV144 vaccinees with the A*02 allele, but only 3% in the vaccinees without A*02. Because cells prefer HLA A*02 to present HIV peptides from the V2 part of HIV's Envelope protein to CD8⁺ T cells, V2-specific CD8⁺ T-cell responses could be responsible for some of the protection observed in RV144. (So far, much of the focus on the modest reduction in risk induced by the vaccine regimen has been on effects associated with non-neutralizing antibodies to the same part of the HIV envelope.)

This is puzzling because CD8⁺ T cells kill infected cells and are therefore not usually thought to prevent infection, but to control the virus in people who are already infected. “The explanation for what happened in RV144 might be more complicated than what we imagine,” said Hertz, but added that Jerome Kim and colleagues recently reported mucosal CD8⁺ T-cell responses in RV144 vaccinees. Such responses might have had a role in preventing infection, since mucosal tissues are the first sites exposed to the incoming virus.

Another explanation, Hertz said, is what immunologists call “cross-presentation:” One arm of the immune system (the cellular CD8⁺ T-cell response) hands over immunogens to stimulate the other arm (the CD4⁺ T-cell activated antibody response). In this case, MHC class I A*02 molecules could bind V2 HIV Env peptides, internalize them, and hand them over to class II MHC molecules, which activate CD4⁺ T helper cells to

stimulate a V2-specific antibody response. Others have recently reported evidence that this kind of cross-presentation is indeed possible, Hertz said.

Systems biology: The genome as crystal ball

Attendees also heard a lot of talks about systems biology, an emerging branch of biology where researchers measure certain parameters of biological “systems” in their entirety to glean insights about the immune system. For example, researchers use so-called microarrays to measure changes in the activity of all genes shortly after vaccination to predict whether a vaccine candidate is likely to work.

A few years ago, Pulendran and his colleagues were the first to report that a signature of gene expression changes a few days after yellow fever vaccination can be used to predict the level of the later adaptive T- and B-cell immune responses to that vaccine.

Those and similar studies on other vaccines have raised the question of whether each signature is specific to the vaccine in question, or if there is perhaps a more general signature that can be used to predict responses to different vaccines. At the meeting, Pulendran reported that signatures that predict responses to the same vaccine class tend to be similar, but differ from signatures predicting responses to a different vaccine class. For example, the signatures that correlate with immune responses to the carbohydrate components of the two meningococcal vaccines are similar to each other, but differ from those that correlate with responses to yellow fever and other live viral vaccines (*Nat. Immunol.* 2013, doi: 10.1038/ni.2789).

Aderem also reported that he has used a “systems” approach to predict vaccine responses in rhesus macaques. He measured gene expression changes two weeks after the animals received a vaccine that contained Gag, Pol, and Nef proteins from the simian immunodeficiency virus (SIV), and used this information to predict lower viral load after SIV challenge one year later with about 85% accuracy. He said he and his team analyzed pairwise combinations of vaccine response genes and used machine learning, where computers learn from repeated analyses of data how to focus on the most relevant information, to limit the data-crunching this entailed.

Systems biologists are also interested in predicting adverse effects of vaccines. For example, almost half of the 374 children who were vaccinated in a trial that tested an inactivated whole H5N1 flu virus vaccine in the 2007/2008 flu sea-

son had fever after the first of two vaccinations and, as a result, the vaccine was not approved by the Japanese health authorities.

Junichi Ito of the National Institute of Biomedical Innovation in Japan described a way to predict whether these flu vaccine recipients would develop fever. He measured the expression of most of the 2,000 known microRNAs (small RNA molecules that regulate gene expression) in serum samples that had been taken before the vaccination from 85 of the children, and found 73 miRNAs that showed a different expression level in the children who developed fever compared with the ones who didn't. These miRNAs can therefore be used as a biomarker to predict whether vaccinees will develop fever in other trials, he said.

Thomas Scriba of the South African Tuberculosis (TB) Vaccine Initiative, meanwhile, has been trying to predict whether people with latent TB infection are likely to develop active TB disease. One third of the world's population, or about two billion people, are estimated to have latent TB infection, he said; of those, about 10% will develop active TB at some point in their lives. Every year, nine million people develop the active disease, and 1.5 million die.

Just why some people come out of latency while others don't is not understood, Scriba said. But microarray analysis, he said, could be used to identify markers that can help predict whether latently infected people will develop active infection, which would make prevention and treatment of TB much easier.

To see if this is possible, Scriba, in collaboration with Aderem and others, used microarray analysis to measure gene expression changes in blood cells taken from 6,363 adolescents with latent TB in South Africa five times over a period of two years. They found that more than 1,200 genes were differentially expressed in 35 people with TB infection who developed TB disease during that time, compared with 70 people with TB infection who didn't develop disease. Scriba said he could use this information to predict the development of active TB six months in advance with up to 80% accuracy.

The accuracy was lower for earlier time points, but still had excellent predictive value up to 18 months before active TB developed, Scriba said. He hopes the analysis could allow prophylactic treatment of high risk groups and enable researchers to preferentially enroll high risk people into efficacy trials of TB vaccines or treatments.

Because the "systems" approach involves measuring everything, researchers are not constrained by their preconceptions of what to

expect, which is why systems biology can also lead to unexpected insights, something that was perhaps best illustrated in a talk by Pulendran.

When Pulendran and his colleagues measured global gene expression changes in response to flu vaccination, they found that the upregulation of a gene called *TLR5* one week after vaccination correlated with the level of the subsequent antibody response to the vaccine. That surprised Pulendran, because *TLR5* is a receptor that senses bacterial flagellin, which is not present in viruses. So, at first, Pulendran and colleagues thought the flu vaccine they were studying might be contaminated with bacteria.

Turned out it wasn't. Further investigation revealed that mice without *TLR5*, or without bacteria in their gut, showed less differentiation of plasma cells, the cells that produce antibodies. This suggests that the sensing of our own gut bacteria by *TLR5* might help induce the antibody response to vaccines, and that things that disturb them, like antibiotics, might be harmful to some vaccine responses.

A few years ago, Pulendran made another surprising observation: When he used microarray analysis to study the immune response to the yellow fever vaccine, he found that early activation of a gene called *GCN2* in the blood was correlated with the magnitude of the later adaptive $CD8^+$ T-cell response to the vaccine (*Nat. Immunol.* 10, 116, 2009). Pulendran and colleagues also showed that *GCN2* was required for the adaptive immune response in mice.

While it was known that the protein encoded by *GCN2* is activated in response to amino acid starvation of cells in response to stress, the link of *GCN2* to the adaptive immune response was not known before. At the meeting, Pulendran reported that he has now elucidated the mechanism of how *GCN2* activates the adaptive immune response: It induces autophagy, in which stressed cells start digesting themselves to generate energy. If this happens in dendritic cells, it enables the cells to better present antigens to $CD4^+$ and $CD8^+$ T cells, which results in a better immune response (*Science* 2013, doi: 10.1126/science.1246829). This mechanism could explain in part why yellow fever vaccine elicits such a powerful immune response.

These studies are an example that systems biology can actually lead to new insights, Pulendran said. "I never knew what *GCN2* was, and no one knew that it could play such an important role in immunity in this way," he said. In his final remarks to the attendees, Rappuoli, a vaccinologist who is head of research at Novartis Vaccines and Diagnostics, agreed. While systems biology has until recently been in a validation phase, in which researchers sim-

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Photograph by J. Adam Fenster, University of Rochester

"Once a Collector, Always a Collector"

One of the world's largest collections of AIDS posters can be found at the University of Rochester. It contains more than 6,200 posters from over 100 countries in over 60 languages, and can be viewed at <http://aep.lib.rochester.edu>. The posters were donated by Dr. Edward Atwater, an emeritus professor of medicine at the University of Rochester. Atwater collected most of the posters between around 1990 and 2005. Andreas von Bubnoff asked him how the collection came together.

How many posters have you collected?

I think there were about 6,500. The University has put 6,200 online but there are some duplicates among them and there are others not yet digitized. Also, I'll soon add another 800 I got from trading duplicates.

Why did you do it?

I am a collector by nature. I started to collect the posters as medical history documents but very soon realized that they were much more important as social history.

When did you get your first poster?

It was probably about 1990. I was riding the Red Line of the Boston subway. I looked up where there are posters in the top of the cars advertising various things. There was a poster with a couple of disembodied hands opening a condom wrapper, and it said, "use one." I was struck by this, because when I went to medical school in Boston 40 years earlier, one could hardly mention contraception or condoms. When we had a lecture on contraception one day, they closed the doors as if the police were about to break in. So I was struck by what an enormous social change had occurred in Boston, which used to be very much more influenced by the Roman Catholic Church. When I came home that day, I called the Public Health Department and asked if they could send me some of those posters. That was the beginning.

So that was quite some time after AIDS was initially reported, wasn't it?

Yes. AIDS was beginning to be recognized in 1981, and it was 1986 that Dr. Koop, the Surgeon General, issued his report on AIDS. That was one of the first times he'd been allowed to discuss it. The Reagan administration had ignored it for five years. The report said you have to either abstain or use condoms. That totally changed the ball game as far as the educational part of using posters and other materials was concerned. It was the Surgeon General's report that blew the lid off things, and you started to see condom posters all over the place. This wasn't just the United States, this was all over the world, even Italy and Spain and countries like that had posters promoting condom use.

When did you collect the posters?

From about 1990 or so, until 2005, or thereabouts. About fifteen years. Today this country



US, 1994. Minnesota AIDS Project. Poster from the AIDS Education Poster Collection of the University of Rochester; poster reproduction by Michael Hager, Museum Photographics.

is no longer producing many posters, I suppose because most people know about AIDS. I won't say there are no posters, but there certainly are many fewer posters. But in other countries they are still published.

I noticed that sometimes the posters don't have the year they were made. Was it hard to find out exactly when they were produced sometimes?

Well, that's one of my great regrets, that I didn't write the date on each poster when I got it, at



Russia, 1990. *Russian: AIDS – casual (sexual) encounters.* Poster from the AIDS Education Poster Collection of the University of Rochester; poster reproduction by Michael Hager, Museum Photographics.

least in the beginning. That wouldn't have been necessarily the date they were made, but it would mean at least that they had been made by that date. Since the subject matter gradually changes, it makes a wonderful historical record; it gives an idea of our evolving understanding of the disease and the attempts to control it. Some of the posters do have dates on them, and I believe the people who are curating the collection at the University of Rochester are planning to add those dates to the online images.

So when do you think most posters were actually made worldwide, and what kind of trends did you see over time?

Between 1986 to 1996, probably. Initially, they started out promoting abstinence, but that didn't sell too well. They also reassured people that they wouldn't get AIDS by sitting on a public toilet seat or eating food in a restaurant. Then, as they discovered what caused AIDS and that there was a test, they started promoting the test. The test made

it possible to reduce the transfusion of hemophiliacs with infected blood. Addicts were taught not to share needles, and to "wash their works." Then it was realized that the proper use of condoms gave some degree of protection and there was—and still is—an enormous output of "use condom" posters. Later, as useful drugs appeared, they were promoted, and with the coming of antiretroviral drugs came a whole new type of poster. There were also posters urging people not to treat people with AIDS or who were HIV positive as pariahs.

How did you get the posters, especially from so many different locations?

I wrote to places and visited organizations, probably 50/50. I started out collecting only US posters, but then I branched out to Canada and then to Europe, because my wife and I went to Europe and we found tons of posters in England, France, Germany, and other countries; Holland put out a lot too. One time we were in East Berlin and we went to an AIDS place there. It was a huge warehouse

just filled with posters—Germany produced a great many posters—and they said ‘Oh, just help yourself!’ So we could just barely get back on the airplane, I had so many tubes of posters. As for the very exotic ones like Bangladesh, the Seychelles, or Africa, friends brought them to me who went there. I also got posters from places like the Center for Communication Programs at Johns Hopkins, or the Canadian Public Health Association, as well as by trading with private individual collectors.

So what countries are least represented in the collection?

While there are a lot of posters from India, Australia and New Zealand, the other countries in Asia and Oceania are relatively rare I think. Asian countries generally, Chinese and Muslim, are the most difficult to come by, Muslim especially. I don’t speak any of those languages, and writing letters to people in Afghanistan or some place like that is not a very practical thing, even if you knew where to write, which I didn’t.

So the kinds of places you got them from were governmental or non governmental organizations that had something to do with HIV messaging, right?

Yes.

And you never had to pay for them?

Oh, occasionally I paid for them. For example, most of the Russian posters I bought from a New York dealer. For those coming from private organizations there was sometimes a charge; if not, I usually made a contribution. Those coming from government organizations were free.

In what kinds of places would the posters actually be posted?

That’s an interesting thing you ask. I never could understand where these posters were used, because I rarely ever saw one posted. But in small communities, for example if you go to the Virgin Islands, there will be a clinic in the town and they will have an AIDS poster or two on the wall. I suppose they were put up in gay bars and bathhouses. And even though it started out—and Mr. Reagan thought of it—as a gay disease, it of course very rapidly became obvious that it was not just a gay disease, and there were posters that were addressed to the non-gay public, but I don’t know where those posters were used. I rarely saw one in use. I have asked others and they agree that they were seldom seen.

Even in the US?

Yes, that’s right. I did see them, but not very often.

What are some examples as to how different countries or cultures handled the disease?

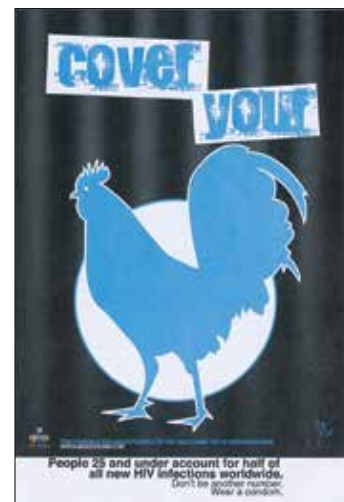
Those differences were what really kept me collecting. It was so fascinating to see how different countries addressed this problem. For example, in Iceland they produced posters that showed a lot of prominent people, the wife of the president, prominent politicians and movie stars, people like that, all doing something silly with a condom to sort of demystify it. Now I don’t think you can imagine such people doing that in this country. A small rural community in Ontario provided one of my favorite (pair of) posters: one of them shows a rooster and the other shows a cat. The one with the rooster says, simply, “cover your,” and the one with the cat says, “protect your.” A rather subtle but explicit message. My children’s favorite is a poster where one is looking through the windshield of a car, and sees a young man and a young woman kissing, and the caption is, “Vanessa was in a fatal car accident last night. Only she doesn’t know it yet.” Every country addressed the problem very differently, even different parts of a country and different groups in a country. But the humorous posters and the political posters are the ones I find most interesting.

What is the most interesting example of cultural differences?

Perhaps the most striking difference is found in the use of humor. France, Holland, Belgium, England, the United States, Canada, and Australia all include quite a bit of humor and double entendre. Germany and Austria, on the other hand, emphasize the explicit. The Soviets produced a lot of arty posters. In Africa, the posters tend to be graphics that illustrate an instructional story. Muslim posters, at least the ones I have seen, are very circumspect, barely mentioning the disease and certainly not condoms. The United States and France, especially, produced quite a few political posters, thanks primarily to the organization ACT UP.

What did the posters really tell you about how people deal with disease?

One thing it tells you is that if you are really in a bind, if you have a uniformly fatal disease, a



Posters from the AIDS Education Poster Collection of the University of Rochester.



US, 1989. ACT UP; Keith Haring poster. Poster from the AIDS Education Poster Collection of the University of Rochester; poster reproduction by Michael Hager, Museum Photographics.

disease without a known cure, then even the most conservative countries or people—in this country Protestant fundamentalists or Roman Catholics—will bow to the use of condoms and talking about sex publicly. AIDS brought sex out of the closet and into the public discourse in a way never previously possible. For example, in the *New York Times* I don't think the word "condom" had appeared more than once or twice in the 50 years prior to the late 1980s, and now you see it every day. People just talk about it as if there is nothing to it. That is a fundamental change.

What's an example for the most conservative societies, judging by the posters in your collection?

I would say Italy or Spain would be considered conservative with respect to talking about sex publicly, but in the AIDS "bind" they talk turkey. China sort of denied that AIDS existed in China (at least at first). South Africa completely denied that it existed. Muslim countries did put out posters but rather discreet posters that were just formal and said don't do it, be careful if you do it.

And what countries would be the most open, judging from the posters?

If by "open" you mean explicit, I suppose the answer is most western European countries, Eng-

land, and the United States, Canada, Australia, New Zealand, Brazil, Mexico. They were more open about sex. They talked about the facts of life. Asian countries (except India) generally less so.

Do the posters tell you anything about design issues and how art is developing?

There are a lot that are good art and some of the great artists of the day did posters—Keith Haring, or the Canadian, Joe Average, for example. A lot of them were done by popular modern artists.

Are there any posters that are just not very well done at all?

Well, the ones our federal government put out were generally not very interesting or clever (with a few exceptions), but they have to be very careful, because they can't afford to offend anyone, whereas private, small organizations can do that and get away with it.

What are you collecting now?

My primary collecting interest is medical history books, pamphlets and broadsides, especially those that are addressed to the public—popular medicine and health reform, but I still collect AIDS education materials as well.

So you are still collecting!

Yes—once a collector, always a collector. ■

Research BRIEFS

An easy-to-use, portable T-cell counter

Earlier this year, the World Health Organization announced new treatment guidelines recommending that antiretroviral therapy (ART) be initiated in HIV-infected adults when the CD4⁺ T-cell count reaches 500—up from the previous threshold of 350. The guidelines pose a considerable challenge because of the limited availability and cost of ART, especially in developing countries.

Another problem is that most HIV-infected people in developing countries don't have easy access to facilities that can measure their CD4⁺ T-cell counts. Currently, such measurements are typically taken with flow cytometers. These large, refrigerator-sized devices weigh around 90 pounds, cost up to US\$100,000, and require trained people to draw blood and do the analysis. One analysis can take several hours.

One reason such devices are so costly is that they use laser light to detect fluorescent antibodies that bind to CD4⁺ T cells. But Rashid Bashir at the University of Illinois at Urbana-Champaign and his colleagues have built a device that lacks optical components and can count all white blood cells, as well as CD4⁺ and CD8⁺ T cells, using plain old electrical current. Better yet, the device can be used by any untrained person—even the patients themselves—to count these cells in less than 15 minutes (*Sci. Transl. Med.* 5, 214ra170, 2013). All that's needed is a drop of blood from a finger prick.

While the current version is just a prototype, the researchers plan to commercially develop a smaller, \$500-\$1000 toaster-oven sized version of their new device in the next 2-3 years. "This approach has the potential to be realized as a handheld, battery-powered instrument that would deliver simple HIV diagnostics to patients anywhere in the world, regardless of geography or socioeconomic status," they write.

To be sure, a portable CD4⁺ T-cell counter is already commercially available. It's a table top device called Pima that uses optical components and is made by a company called Alere. And Bashir himself has cofounded a company called Daktari Diagnostics to commercially develop an electronics-based portable device that will be tested in Africa.

But the new device, once commercially developed, could be

the first electronics-based handheld portable device that can also count all white blood cells and CD8⁺ T cells, in addition to CD4⁺ T cells. That's important, Bashir says, because doctors can use the total white blood cell count and the CD4/CD8 ratio to determine the status of a patient's immune system and to better monitor whether their treatment is working.

The way the new device counts cells is quite simple: It measures how an electrical current changes as cells pass through a group of tiny electrodes. Antibodies are then used to capture CD4⁺ or CD8⁺ T cells, and the cells are counted again. The difference between the two counts equals the number of captured CD4⁺ or CD8⁺ T cells. What makes the device so affordable, says Bashir, is the lack of optical components and the fact that all measurements and biochemical reactions take place on a chip that is similar in size and composition to a computer microchip.

Because the device can be used by any untrained person, it should make CD4/CD8 counts much more accessible to people in the developing world. "[People] just can't make it to the labs," Bashir says. "This technology can take the lab to the patients rather than the patients to the lab, so a case worker can go to very remote settings and do the test right there in the field."

But not everyone thinks that CD4⁺ T-cell counts will remain as important in the future as they are now. Some researchers say that offering treatment at any CD4⁺ T-cell level (an approach called Test and Treat) is the right way to go because there is evidence that the earlier HIV-infected people start treatment, the better. Julio Montaner, who runs a Test and Treat program in British Columbia, says the study is very good news. "However," he adds, "as we move towards a full Test and Treat strategy (hopefully in the very near future), the need for CD4 cell count monitoring decreases sharply."

But Bashir is already thinking of other applications. Because the technology can in principle be developed to count any type of cell that's recognized by a specific antibody, he plans to develop a version that can replace flow cytometers in performing a very common test that's done pretty much every time someone gets a physical: the complete blood count, which measures most of the types of cells found in the blood. —*Andreas von Bubnoff*

Continued from page 13

ply confirmed things that were already known, he said, Pulendran's results show that the field is ready to discover new things.

He ended the meeting with a glimpse of the future. Systems biology, he said, might eventually help accelerate the development of candi-

date vaccines—which, he noted, can take about 15 years to move from bench-top to clinic. "That's a long time," he said, adding that while clinical trials today test, say, 10 parameters in 10,000 people, systems biologists might be able to find ways to do the same thing with

much less effort. "I want to leave you with [a] dream for vaccine development," he said. "If we could use [this] technology [to test] ten people [with] 40,000 data points per person to predict what's going to happen, probably vaccine development would be much faster." ■

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