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The Publication on AIDS Vaccine Research

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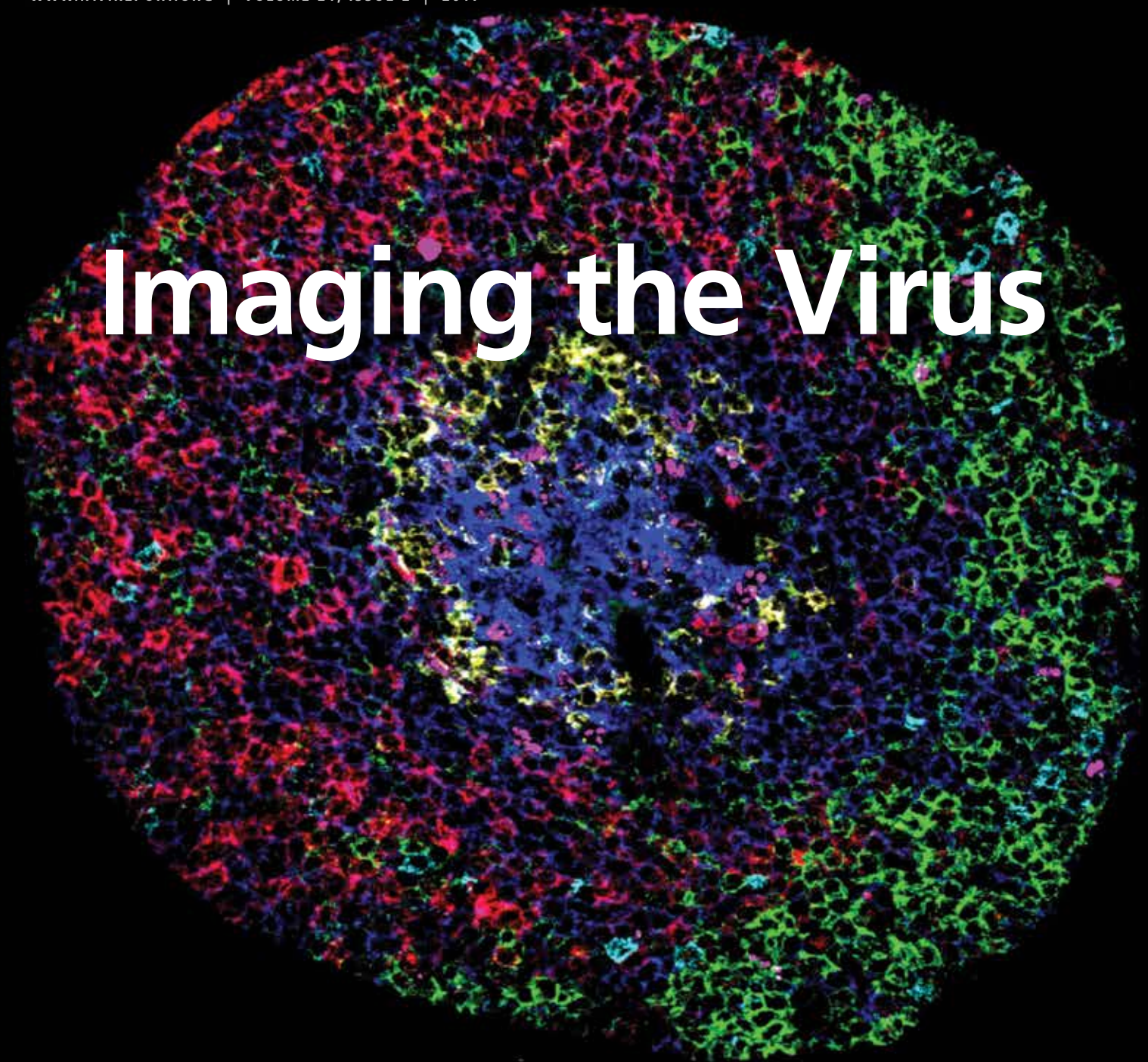

Imaging the Virus

Readying the CMV Vector for Human Trials

Antibody Science from Keystone

Waging War against Future Epidemics

EDITOR'S LETTER

I recently found myself walking past the New York City AIDS Memorial. It is located in a triangular park astride what was formerly St. Vincent's Hospital in the West Village neighborhood of Manhattan. The centerpiece of the Memorial is an 18-foot geometric white slatted metal structure that stands over a black fountain, around which is marble engraved with text from Walt Whitman's "Song of Myself" from *Leaves of Grass*. The text component was created by artist Jenny Holzer, and according to Wikipedia, more than 500 architects contributed to the design of the memorial that officially opened on World AIDS Day (December 1) 2016.

It is a stark and chilling reminder of the city's more than 100,000 men, women, and children who have died of AIDS. Yet as you glance up at the sky through the canopy's triangular structure, you can't help but feel hopeful. "Do I contradict myself? Very well then I contradict myself, (I am large, I contain multitudes.)" Whitman's line ends the string of text at the memorial and seems an appropriate way to summarize the emotions it invokes. AIDS is large. It contains multitudes. And there are still many stories to tell.

In this issue, we have stories running the gamut from scientific updates presented at the *Keystone Symposium on HIV Vaccines*, held in March (see page 4), to a profile of the promising cytomegalovirus-based HIV vaccine candidate that is soon to enter Phase I clinical trials (see page 14).

On the broader issue of pandemic preparedness, I spoke with Michael Osterholm, author of the recently published book *Deadliest Enemy, Our War Against Killer Germs* (see page 19). In his book, and throughout our interview, he provides a lucid and somewhat terrifying description of the top infectious disease threats facing the world and how more science and funding are needed to keep them at bay.

Finally, if a picture is worth a thousand words, then the recently held workshop, *Harnessing Novel Imaging Approaches to Guide HIV Prevention and Cure Discoveries,* was probably akin to Proust. This two-day meeting featured a slew of interesting pictures of the virus, and two of the meeting's co-chairs curated a selection of the best for us to feature in this issue (see page 9). These useful, and dare I say beautiful images, are allowing scientists to visualize the virus's interactions with the immune system in an attempt to create better preventive and cure strategies.

"Do you see O my brothers and sisters? It is not chaos or death—it is form, union, plan—it is eternal life—it is happiness. The past and present wilt—I have fill'd them, emptied them, and proceed to fill my next fold of the future." —Walt Whitman, "Song of Myself"

– KRISTEN JILL KRESGE

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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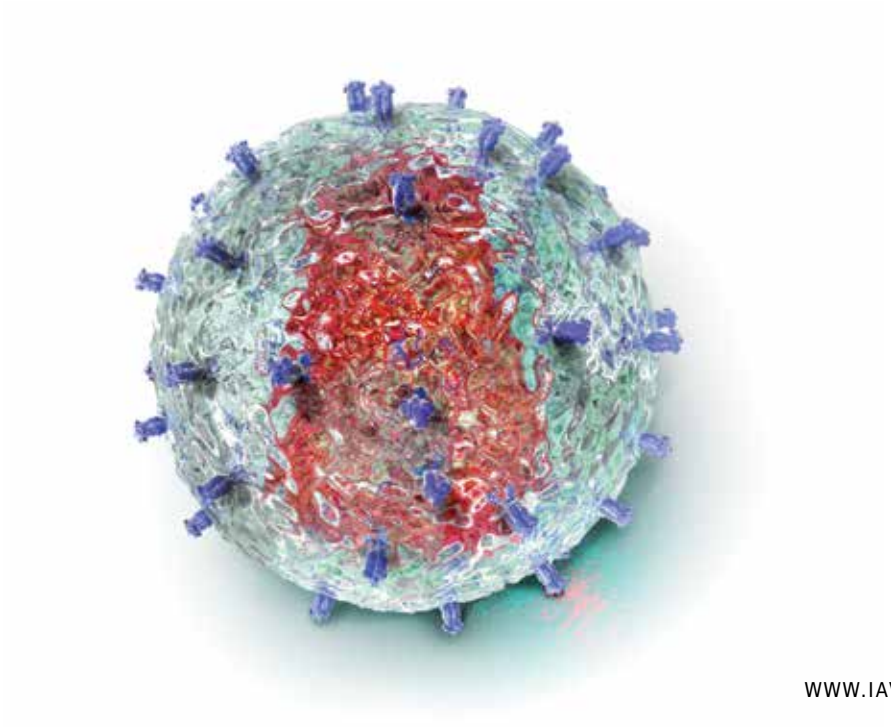

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SENIOR PRODUCTION MANAGER
Nicole Sender

MANAGING EDITOR
Kristen Jill Kresge

CONTRIBUTING WRITERS
Michael Dumiak
Morgane Rolland
Mary Rushton
Yegor Voronin

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[ON THE COVER]

This is a tonsillar B-cell follicle labeled with antibodies against IgD (red), CD20 (blue), CD4 (green), CD8 (cyan), PD-1 (yellow), and Ki67 (pink).

Image courtesy of the Tissue Analysis Core, Vaccine Research Center, NIAID, NIH.

Protein Progress

Like the development of the antibodies themselves, understanding and optimizing immunogens designed to elicit a broadly neutralizing response against HIV takes time.

By Morgane Rolland and Yegor Voronin

***Morgane Rolland** is Chief, Viral Genetics Section, US Military HIV Research Program, Walter Reed Army Institute of Research; The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.*

The views expressed are those of the author and should not be construed to represent the positions of the U.S. Army or the Department of Defense.

***Yegor Voronin** is senior science officer at the Global HIV Vaccine Enterprise.*

In science, results often come slowly. Vaccine research is a painstaking process and sometimes it can be hard to tell whether progress is being made at all. But the results presented at the Keystone Symposium on HIV Vaccines, held in Steamboat Springs, CO, March 26-30, are unambiguous—researchers are making strides in their attempts to develop antibody-based vaccine candidates. Thanks to continuing efforts to learn how broadly neutralizing antibodies (bNAbs) are generated during natural infection and application of this knowledge to design and test novel immunogens, there is a wealth of promising results and discoveries to report.

Responses induced by trimeric proteins

One major effort to develop bNAb-based vaccine candidates relies on presenting the immune system with an HIV protein that mimics the natural structure of the virus's trimeric Envelope protein[1]. These native-like Env proteins (including the SOSIP family of native-like trimers and related approaches; see *Many Keys to Protection but Many Locks Remain*, *IAVI Report,* Vol. 20, Issue 4, 2016) have not yet been tested in humans, but several groups are studying them in various animal models[2,3].

At Keystone, Andrew Ward, associate professor at The Scripps Research Institute (TSRI) in La Jolla, CA, synthesized the lessons learned from several independent studies of antibodies isolated from trimer-vaccinated rabbits and macaques. Antibodies to autologous viruses (those that carry the exact versions of Envs that were used for immunization) were observed at different levels across studies. The antibodies were primarily able to block the easier to neutralize Tier-1 viruses, with a few native-like trimeric proteins eliciting antibodies to the harder to neutralize Tier-2 panel of viruses.

Some of the trimers evaluated to date have chinks in their glycan armor, characterized by glycans missing in key spots on the surface. These so-called "glycan holes" were targeted by antibodies elicited in response to these trimeric immunogens in multiple animals[3,4]. In some cases these responses led to effective neutralization of autologous viruses. But it remains unclear whether a vaccine strategy based on glycan hole-targeting antibodies would lead to the development of a broad antibody response targeting multiple viral variants, which is what would be required to fend off the broad spectrum of viruses in circulation, or whether it will lead to a potent, but narrowly focused response. Data from an *in silico* analysis presented by Kshitij Wagh, a staff scientist at the Los Alamos National Laboratory, indicated that glycan holes have a negative effect on the development of neutralization breadth and that focusing immune responses on glycan holes may favor strain-specific antibodies at the expense of a broader antibody response. Ward pointed out that the first of the constructed native trimers, BG505 SOSIP.664, is missing two glycans at positions 241 and 289. Presence of the glycan 289 would completely block binding of antibodies targeting this "hole" and this glycan is found on a substantial proportion of viruses around the world, thereby making them resistant to the glycan hole-focused antibodies induced by this trimeric protein.

Other SOSIP-elicited antibodies discussed at Keystone targeted the gp120/gp41 interface, fusion peptide, V3, and V1/N332 supersite, often in a similar manner in multiple animals (see Figure 1, page 5). These are promising results because they are reminiscent of responses found in natural HIV infection, including those that lead to the development of bNAbs in a sub-

set of chronically HIV-infected individuals. However, some antibody responses were not representative of what has been seen in people, such as those targeting the base of the soluble trimer that normally faces the lipid membrane of the virion and is not easily accessible by antibodies[5]. The consequences of these off-target responses need to be further evaluated to understand whether they can be ignored or will need to be silenced so as not to distract the immune response from the more critical spots of weakness on the viral Envelope.

Researchers are also exploring ways to augment the responses induced by trimeric protein immunogens. Data presented by Colin Havenar-Daughton, a scientific associate in Shane Crotty's laboratory at the La Jolla Institute for Allergy and Immunology, showed that rhesus macaques generated strong immune responses to native-like trimers administered along with the adjuvant iscomatrix. He highlighted results from Dennis Burton's laboratory at TSRI showing that despite the presence of glycan holes on the BG505 SOSIPs, the majority of responses in macaques targeted other parts of the trimers and were variable in specificity and magnitude.

Havenar-Daughton also presented an innovative way to follow the development of antibody responses that may help explain this variability and possibly suggest ways to make the induced immune response more reproducible. The approach involves inserting a very fine needle into a lymph node in a vaccinated animal and then aspirating a small sample of germinal center cells[6]. Curiously, Havenar-Daughton found a higher number of B cells as well as higher ratio of B cells to helper T cells in the germinal centers from aspirates collected early in the immunization schedule strongly correlated with neutralizing titers observed after the second or third dose of vaccine. This technique should enable investigation of antibody responses almost in real-time, and should therefore help to uncover the underlying mechanisms responsible for the development of antibody responses, their maturation, and the eventual development of neutralization breadth that researchers are hoping to induce through vaccination.

A combination approach

Despite the current focus on native trimers, many researchers feel they represent only one piece of the puzzle that will have to be put together to create a vaccine that can induce bNAbs. To this end, Peter Kwong, senior investigator at the Vaccine Research Center (VRC) at the US National Institute of Allergy and Infectious Diseases, presented results from studies testing a combination of SOSIP trimers with a scaffolded immunogen in a prime-boost regimen. The scaffold is designed to present an eight-residue peptide that corresponds to the N-terminus of the fusion peptide (FP), a conserved region of gp41 targeted by some bNAbs. Kwong and colleagues tested a wide variety of immunization strategies in mice and identified an approach that resulted in neutralizing responses, with some antibodies neutralizing select Tier-2 strains of HIV from subtypes/CRF A, AE, B, BC, and C. Optimal immunization required priming with a trimer, boosts with FP-carrying scaffolds, and an additional boost with a trimer. To improve on this result researchers are optimizing the immunization strategy on two fronts. First, they are testing various versions and combinations of the FP peptides to improve the breadth of the immune responses. Second, they are comparing different scaffolds, some of which appear to be far more immunogenic in mice than the scaffold that was used in this study. The long-term objective of this work is to reproducibly elicit antibodies that neutralize at least 30 percent of the Tier-2 strains of HIV.

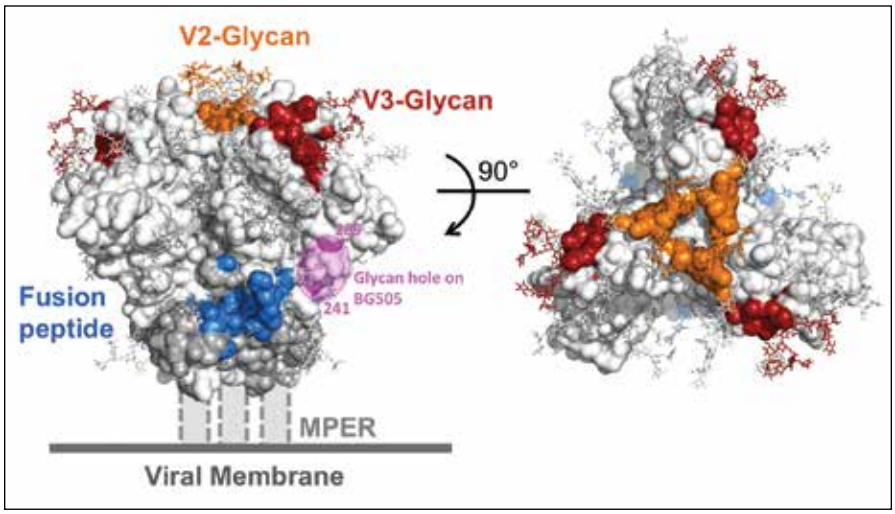
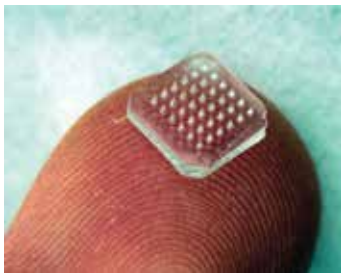


Figure 1. HIV Env trimer. Fully glycosylated crystal structure of BG505 SOSIP.664 Env trimer (side view on left and top view on right) with Env-gp120 colored in white and Env-gp41 in gray. Key Env sites mentioned in the article are shown here with the epitope sites corresponding to representative antibodies: V2-Glycan (PG9), V3-Glycan (PGT122), and fusion peptide (PGT151). The glycan hole created by the absence of glycosylation at amino acid sites 241 and 289 is represented in pink. Image courtesy of Hongjun Bai, US Military HIV Research Program, Walter Reed Army Institute of Research.



A patch containing 36 dissolving microneedles is shown on a fingertip. The microneedles dissolve within minutes after insertion into skin to release encapsulated drug or vaccine. Each microneedle is 900 µm tall. Credit: Jeong-Woo Lee, Georgia Tech

Ryan Meyerhoff, an MD/PhD student at Duke University, presented results from Bart Haynes' group that also pointed to the need to combine trimers with immunogens that focus responses on a particular part of the Env protein. Their work makes use of a synthetic glycopeptide mimicking glycans on the V3 region[7,8] of HIV Env, targeted by such bNAbs as 2G12 and PGT125. Immunizations with a polymeric version of this peptide resulted in neutralizing responses in all four of the immunized macaques. Isolation and analysis of antibodies induced in these animals showed that they targeted the V3 region in a similar manner to V3-targeting bNAbs, by recognizing both the N301 glycan as well as the GDIR amino acid motif, which corresponds to the tip of the V3 loop (amino acids 312-315). When boosted with a SOSIP trimer, these antibody lineages greatly expanded and showed evidence of evolution, presumably to acquire enhanced binding to the native envelope. Further analysis of these responses is underway, as well as efforts to design better boosting immunogens that would select for greater breadth of antibody responses.

Presenting proteins

Meanwhile researchers are also exploring novel ways of presenting these immunogens to enhance the immune response. Paola Martinez-Murillo, a PhD student at Karolinska Institutet, presented results from Gunilla Karlsson Hedestam's group showing that arranging native-like clade C trimers on liposomes improved the quality of the immune response as observed by the formation of larger germinal centers that contained more $CD4^+$ T follicular helper (Tfh) cells than observed after immunization with soluble trimers. The liposome-arrayed trimers also elicited stronger and more consistent autologous Tier-2 neutralizing antibody responses. By sorting Env-specific memory B cells and isolating a set of monoclonal antibodies, the investigators showed that the autologous Tier-2 neutralization was mediated by antibodies that target the V2 cap region of the trimeric Env spike[9].

Darrell Irvine, a professor at the Massachusetts Institute of Technology, is taking a very different approach by putting SOSIPs into microneedle patches—arrays of tiny cone-like needles that pierce the outermost layer of the skin and deliver their content intradermally by dissolving at a defined rate (see image, this page)[10]. His lab has previously shown that a sustained, or even escalating, release of antigen is better at stimulating antibody responses than a single shot. The same was true in a head-to-head comparison of SOSIPs delivered by either microneedles or traditional soluble protein in mice. With the use of microneedle injection there was a quantitatively stronger response, with an increased frequency of Tfh cells in germinal centers and, most importantly, approximately 100-fold higher titer of Env-binding antibodies after immunization. An additional benefit of microneedles is that they are made from non-immunogenic silk protein used in dissolvable sutures. The protein protects SOSIP structures during lyophilization, removing the need for cold chain storage.

These and other studies presented at the meeting illustrate the extensive analyses that native-like trimers are undergoing in animal models, both in search of a better understanding of the specific responses being activated by these immunogens and also for empirical investigation of candidate Env vaccines and immunization regimens that result in higher neutralization titers and wider breadth of response in animals and ultimately in humans.

bNAb development in natural infection

Studies of antibody development in HIV-infected adults have revealed a number of important concepts that inform vaccine development. An important observation is that bNAbs bind to different epitopes on HIV Env proteins, suggesting there are multiple targets for vaccine researchers to pursue. Another is that development of bNAbs usually takes years. And, as a result of continuous exposure to perpetually mutating viruses, bNAbs often have very high somatic hypermutation rates, suggesting that co-evolution of antibody lineages and viruses is dynamic over a period of several years.

To create vaccines that would be widely applicable, it is therefore important to better understand bNAb development in different contexts. Only a few studies have thoroughly characterized the development of bNAb responses over time in infected individuals, so additional studies are needed to find how generalizable these findings are. Identifying shared patterns of bNAb development involving different antibody epitopes, viral subtypes, and ethnic groups would pave the way toward developing a broadly applicable vaccine. As such, the Keystone symposium highlighted a variety of approaches—from the large-scale neutralization breadth analysis of more

than 4,500 individuals in the Swiss cohort to the minute analysis of bNAb development in select individuals, as well as the comparison of bNAb development in infants versus adults.

Alexandra Trkola, a professor at the Institute of Medical Virology at the University of Zurich used data from the Swiss 4.5K Screen, a large cross-sectional analysis of HIV neutralization activity in two longitudinal Swiss cohorts that included close to 4,500 individuals, to further evaluate the association between bNAbs and host, virus, and disease characteristics. In a recent study the group identified four factors that contribute to the development of broad responses[11]. One host characteristic was identified: individuals with black ethnicity showed greater neutralization breadth. And three factors linked to the virus—higher viral load, longer infection length, and a higher degree of viral diversity—were associated with greater neutralization breadth. They then looked at the binding antibodies using 13 HIV antigens and obtained immune signatures linked with the same four determinants that were found for development of neutralization breadth. Emerging data gathered from studying more than 300 HIV transmission pairs showed that viral characteristics explain up to 15 percent of the variation in development of neutralization breadth between individuals, providing the first delineation of the contribution of host versus viral factors in the development of a broadly neutralizing antibody response.

Additional studies of antibody development in specific individuals can also complement large-scale studies such as that being pursued by Trkola. One example of studying a single HIV-infected volunteer came from Elise Landais, a senior scientist at the International AIDS Vaccine Initiative (IAVI). She presented data from an individual in an IAVI cohort in whom viral diversity, as suggested by the Swiss cohort data, appears to be implicated in the development of neutralization breadth[12]. Understanding the ontogeny of bNAb responses in a given subject provides new ideas for vaccine design.

While there is not currently a study comparing the development of bNAbs in different individuals against a common Env epitope, this knowledge gap will likely narrow in the coming years as multiple studies in different cohorts are underway. Nicole Doria-Rose, a staff scientist at the VRC, traced virus-antibody co-evolution in an HIV-infected individual from the US Military's HIV Research Program's (MHRP) RV217 cohort and thereby discovered for the first time a pathway toward a membrane proximal external region (MPER)-directed bNAb. Meanwhile, Penny Moore, an associate professor at the National Institute for Communicable Diseases in Johannesburg, South Africa, presented follow-up analyses on the CAP256.VRC26 antibody lineage, which targets the V2 Env region, and is possibly the most extensively studied case of virus/antibody co-evolution[13]. Moore and colleagues aimed to understand why some neutralizing antibodies within a lineage become bNAbs, while others retain narrow specificity despite equally high levels of somatic hypermutation. The CAP256.20 antibody has narrow specificity, while the CAP256.27 antibody is broadly neutralizing. Importantly, introducing three mutations found in CAP256.27 into CAP256.20 restored breadth for CAP256.20. The original amino acids at these sites in CAP256.20 allowed the antibody to bind to a virus variant that was common in the CAP256 donor, but was globally rare. This explains the lack of breadth of the CAP256.20 antibody and shows that antibody maturation during infection does not necessarily result in neutralization breadth.

While all these findings come from studies of adult samples, Julie Overbaugh, a member at the Fred Hutchinson Cancer Research Center, summarized findings on infant antibody responses. Previously, her lab has shown that approximately two thirds of HIV-infected infants develop bNAbs very rapidly, within 11 to 24 months after infection[14]. At Keystone, Overbaugh and her lab presented detailed analyses they conducted of antibodies from two infants. These studies confirmed previous findings and also led to some unexpected and even puzzling observations. All of the neutralizing antibodies, including one bNAb, isolated from one of the infants were able to bind the founder virus that established the infection in that child but did not neutralize it. The antibodies did, however, neutralize viruses that appeared three months after infection. Similar results were obtained in a second infant, in whom antibodies that neutralized heterologous viruses did not neutralize the transmitted virus of that infant. This suggests that the antibodies that recognize the transmitted virus seem to be distinct from those that are responsible for breadth. This dichotomy between binding and neutralization, as well as viral escape from binding antibodies, has not been well documented in adults, although there are examples of

it, such as the one from Penny Moore's lab in which CAP256 lineage neutralizing antibodies retained their binding to escape viruses that were no longer neutralized by them.

Unlike in adults, in whom neutralization often depends on a single dominant antibody lineage, plasma mapping studies and detailed study of antibodies from one infant suggest that infant responses appeared to be polyclonal and simultaneously target multiple sites of vulnerability on the envelope, thus attaining breadth of coverage. Moreover, studies of the bNAb isolated from an infant showed that in contrast to bNAbs from adults, which usually show an unusually high level (15 percent to 30 percent) of somatic hypermutation, an infant bNAb was only 7 percent mutated, indicating a more rapid path to neutralization breadth[15]. More studies are needed but the polyclonal nature of the response and the easier path to neutralization suggest that infants may have a particularly favorable immune environment for bNAb-generating vaccines.

All these studies provide a window to the ontogeny of breadth for humoral responses. With a detailed understanding of the viral and host parameters responsible for a strong, potent, and broad antibody response, researchers can design and develop improved vaccine strategies. In parallel, the field is focusing on optimizing vaccine constituents and regimens to find the best approach to elicit an effective vaccine-induced immune response.

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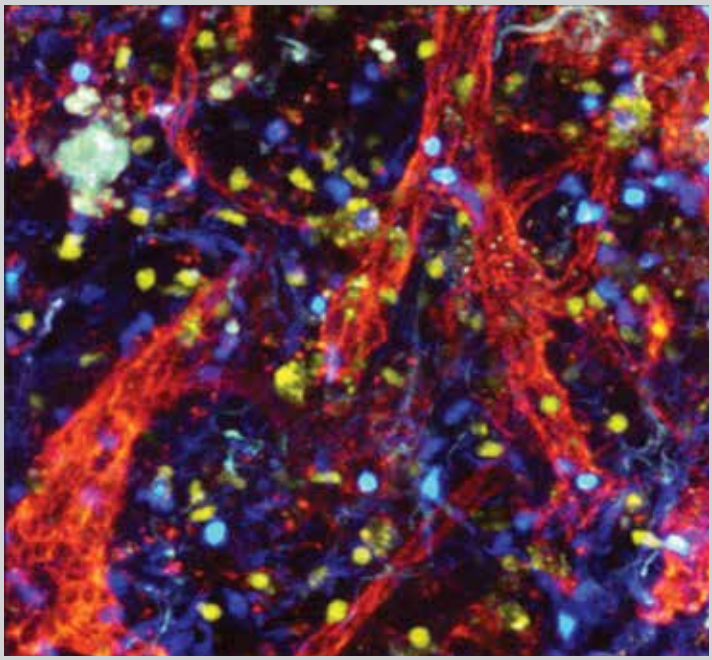
A Picture is Worth A THOUSAND WORDS

Viruses are fascinating on many levels. One of them is visually. Scientific images of viruses, HIV included, can be quite arresting and often blur the lines between science and art (see *The Trimer Transformed*, *IAVI Report*, Vol. 19, Issue 1). At the two-day workshop, "Harnessing Novel Imaging Approaches to Guide HIV Prevention and Cure Discoveries," many of these striking and important images were on display. This meeting was sponsored by the Division of AIDS at the US National Institutes of Health (NIH) and the Global HIV Vaccine Enterprise. *IAVI Report* invited two of the workshop's co-chairs, Constantinos Petrovas of the NIH and Jake Estes of the Frederick National Laboratory for Cancer Research and Leidos Biomedical Research Inc., to curate a selection of some of the top images from the meeting and to describe how scientists are using these images to guide the development of new strategies to prevent HIV infection or even help identify an effective cure. Oh, and they are rather stunning too. —*Kristen Jill Kresge*

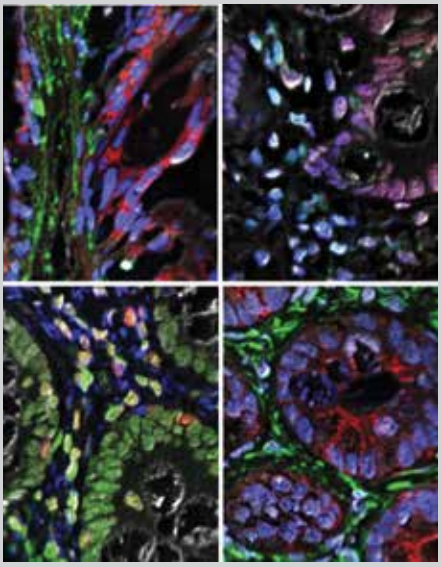


Tim Shacker
Imaging T-cell Motility

This image, created using *ex vivo* two photon confocal imaging techniques, shows T- and B-cell mobility with respect to fibrotic lymphoid tissue damage. This technology allows for the real-time analysis of immune cell interactions within their natural tissue environment. $CD4^+$ T cells (orange) and B cells (blue) were incubated on top of HIV-infected lymph node biopsy slices. Image generated by Jason Mitchell and Brian Fife.

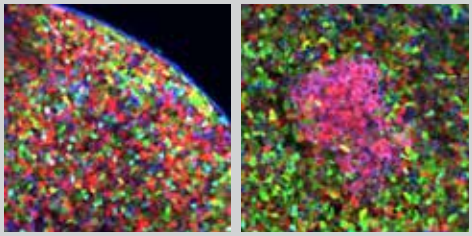
Curating a Conference

The aim of the workshop was to bring leading HIV and non-HIV imaging experts together to discuss cutting-edge technologies and approaches in this field to promote innovation, collaboration, and acceleration of scientific progress in HIV prevention, pathogenesis, and cure research. Novel imaging platforms that allow for the visualization and multi-dimensional and multi-parametric quantitative analysis of the virus at the cellular, tissue, and organism level were presented. The presentations covered a broad range of *in vitro*, *ex vivo*, and *in vivo* imaging approaches for a comprehensive analysis of the pathology of the virus and the immune cell dynamics involved in the interplay between the virus and the immune system. The emerging technologies presented are being applied to understand HIV/simian immunodeficiency virus (SIV) transmission and mechanisms of prevention, the spatial micro-anatomy of the immune system, the biology of HIV/SIV reservoir formation and viral persistence, and disease-driven pathology, leading to the development of new concepts and strategies to eliminate the virus. —*Constantinos Petrovas and Jake Estes*



Michael Angelo
Multiplexed Ion Beam Imaging

The use of spectrometry-based imaging technologies, such as that used here, opens new avenues for in-depth analysis of complex cell populations in their native tissue environment. Multiplexed ion beam imaging (MIBI) uses antibodies tagged with elemental mass tags in combination with secondary ion mass spectrometry to visualize dozens of proteins simultaneously in a single tissue section. Examples of MIBI data are shown as color overlays.

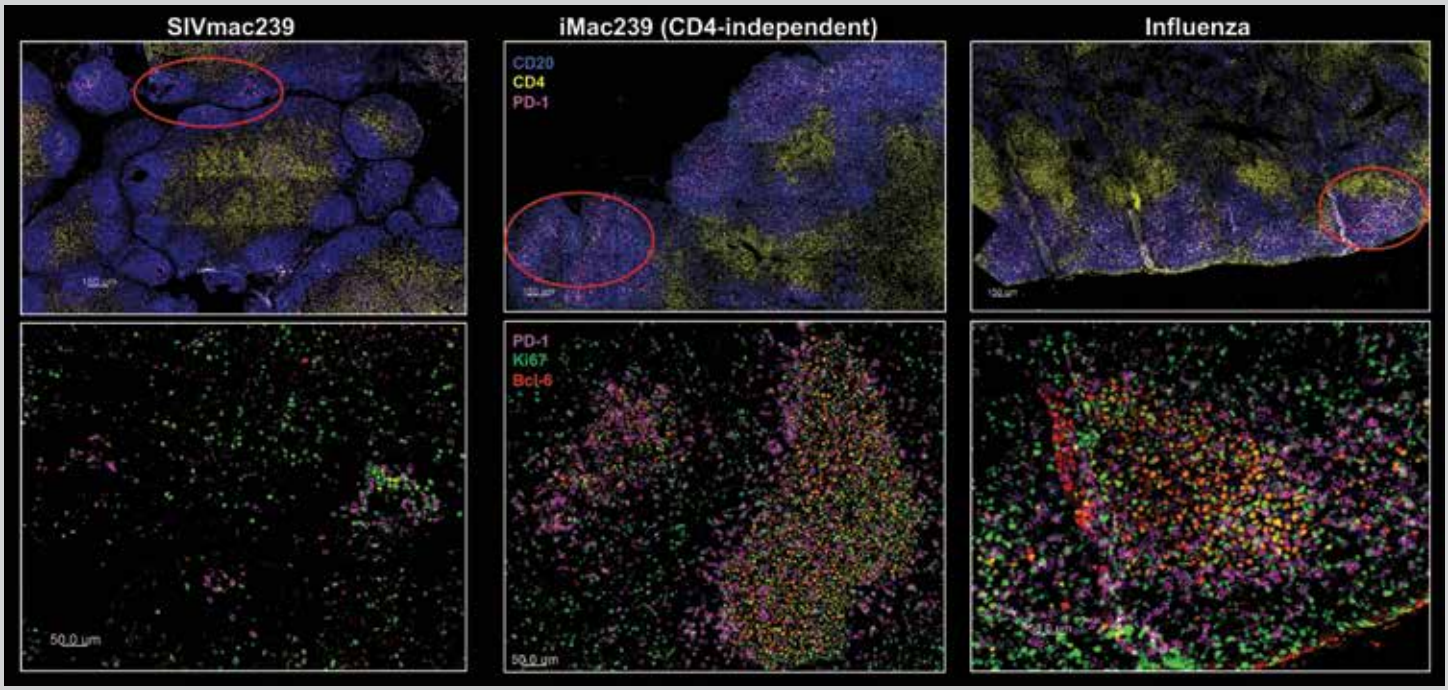


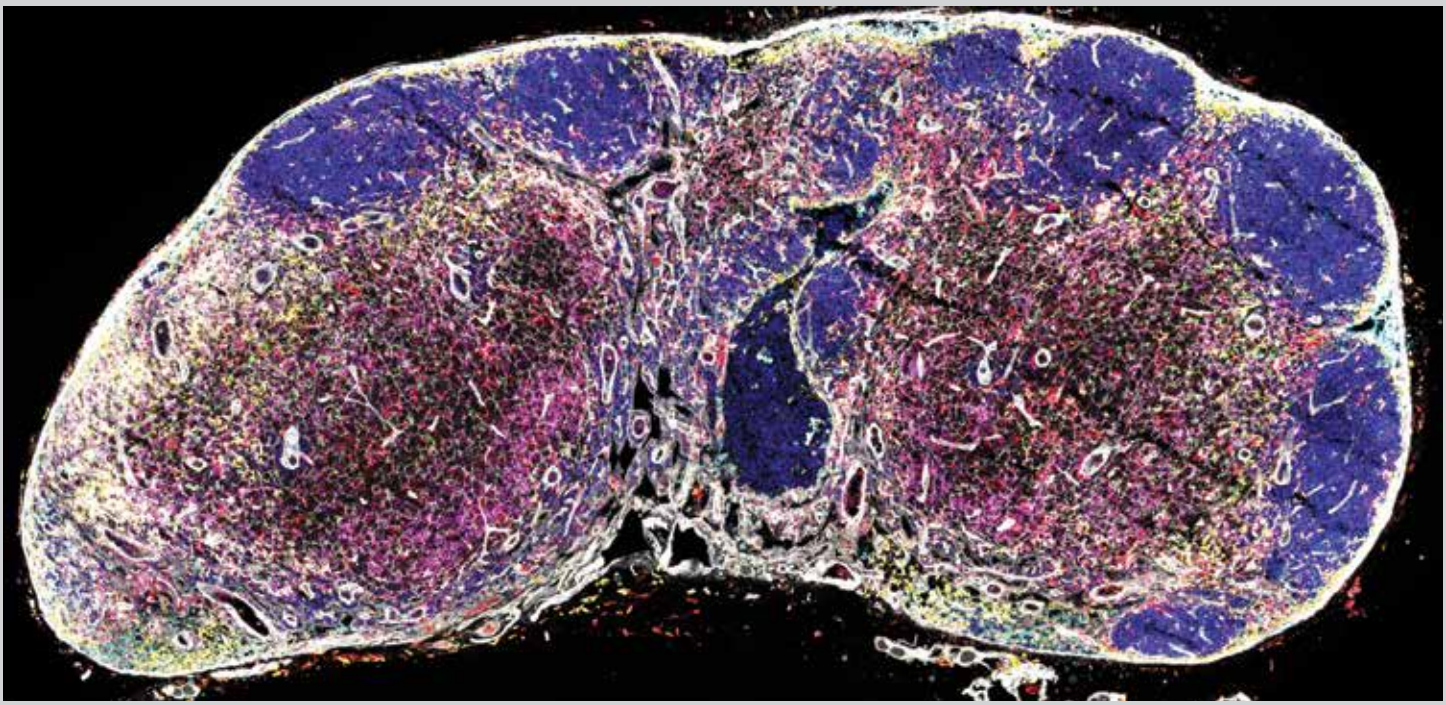
Gabriel Victora
Germinal-center Formation

This image demonstrates how novel mouse models provide unique opportunities for *in vivo* visualization of immune system dynamics and delineate the complexity of the development of immune responses. Image on left, taken using multiphoton microscopy, is pre-immunization. Imagine on right, post-immunization, shows germinal center formation in a mouse lymph node.

Constantinos Petrovas
Infection-induced Germinal Centers

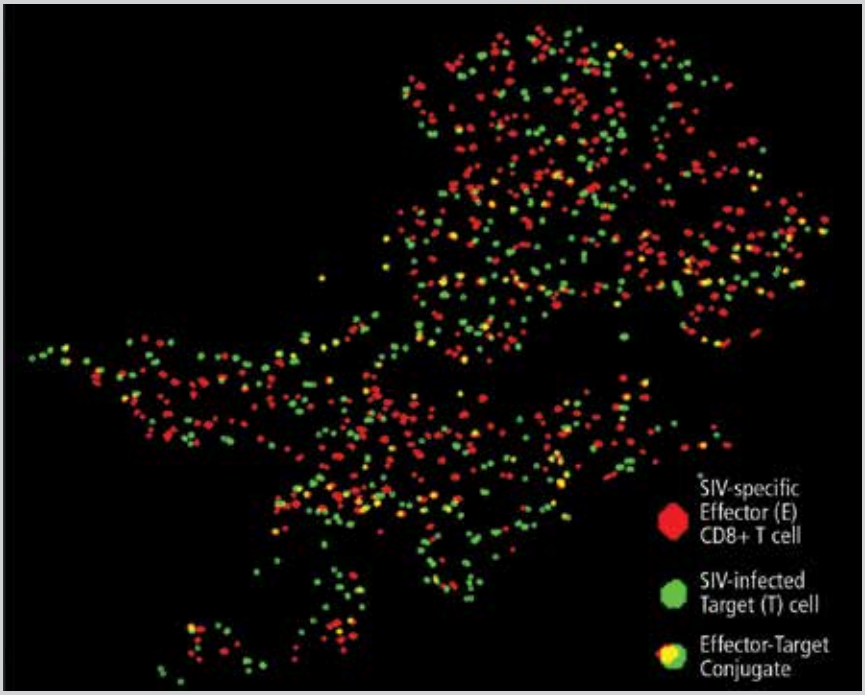
Researchers applied multiplexed confocal imaging assays for the simultaneous quantitative analysis of several relevant immune cell types that mediate the development of pathogen/immunogen-specific B-cell responses. Non-human primate lymph nodes obtained 14 days after infection with wild type (SIVmac239) or a CD4-independent (iMac239) SIV are shown. Germinal centers (defined by CD20 in blue) and follicular CD4$^+$ T cells (defined by CD4 in yellow and PD-1 in pink) with respect to expression of Bcl-6 in red and Ki67 in green are shown.





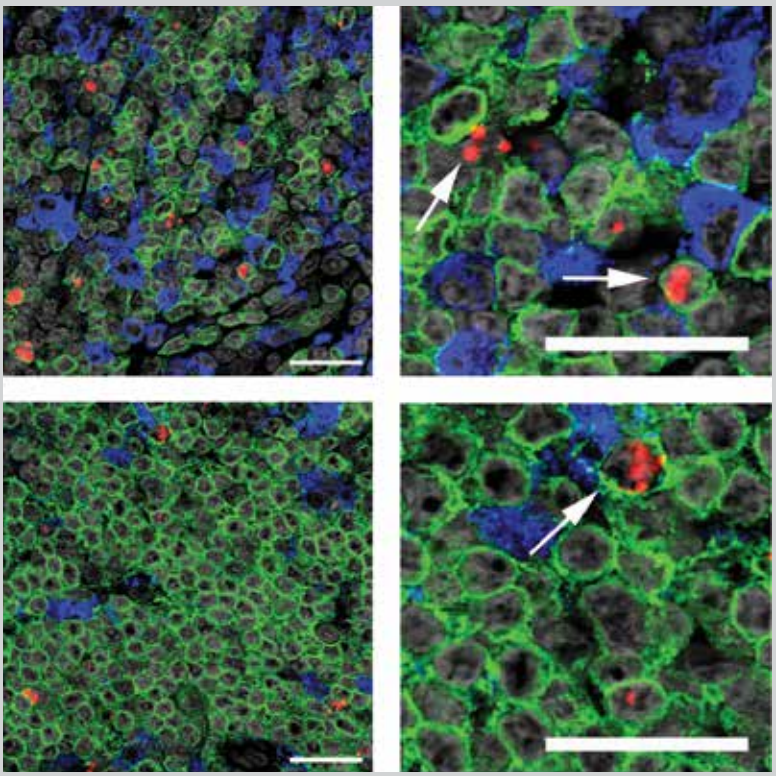
Michael Gerner
Antibodies in Mouse Lymph Node

The multi-dimensional high-resolution confocal microscopy analysis employed to create this image provides critical information for the understanding of the local organization and compartmentalization of relevant cells during the development of immune responses in a mouse lymph node. This helps researchers understand the underlying processes involved in inflammation and the immune response. This image shows a mouse lymph node stained with antibodies to detect various innate and adaptive immune populations, as well as stromal structural elements.



Ashley Haase
Battlefield Map

The work by Haase and colleagues allows us to visualize the local dynamics of SIV infection and the adaptive immune responses soon after infection, showing that the mobilization of the immune system is "too little, too late." While there are many T cells in spatial proximity to infected cells, the infected cells are numerous and the target to effector cell ratio was correlated with only partial control of infection.



Jake Estes
Tracking the Virus in Tissue

This image showcases the power and utility of a novel imaging technology that allows for the detailed characterization of HIV-infected cells *in situ*. This technique should provide critical information regarding virus dissemination, establishment of latency, and the cellular populations and anatomical sites where virus and infected cells persist. Viral DNA is shown in red with $CD3^+$ T cells in green and $CD68^+/CD163^+$ myeloid lineage cells in blue. The arrows point to examples of "superinfected" T cells that contain multiple copies of viral DNA per cell.

Gates Research Institute to Focus on Accelerating Translational Research

Since its inception in 2000, the Bill & Melinda Gates Foundation (BMGF) has spent billions of dollars on global health research, largely by funding academic laboratories and product development partnerships (PDPs). Going forward it will take the unusual step of doing some of the research itself.

A press release posted on BMGF's website on April 25 says they plan to establish a non-profit medical research institute aimed at improving the pace of translational research—that is, the process of turning scientific discoveries into actual products. The release says the institute will focus on "capitalizing on research breakthroughs and identifying viable candidates for drugs, vaccines, diagnostics, and medical devices." BMGF anticipates that the institute will be co-located in Seattle and Boston and that Penny Heaton, who heads up BMGF's Vaccine Development and Surveillance Program, will be taking a senior leadership role in the new institute, according to the press release.

The research institute will receive US$100 million annually from BMGF to study diarrheal diseases, malaria, and tuberculosis (TB). Ambitious projects are underway to eradicate these diseases, but there is still no vaccine to prevent malaria, diarrheal diseases remain a leading killer of young children in developing countries, and better vaccines and drugs are needed in the TB fight, as are more efficient ways of confirming its diagnosis.

Exactly why BMGF decided to switch gears and open its own research institute is unclear. Bryan Callahan, a spokesman for BMGF, told *IAVI Report* that they preferred not to comment until October, when the concept and design of the institute should be complete.

Another open question is how the new research institute will impact PDPs that are working on these same diseases and currently receiving financial support from BMGF. Peter Hotez, director of the Texas Children's Hospital Center for Vaccine Development, says not enough is known now to determine what kind of impact the new research institute will have.

In a Q&A, BMGF said it is still evaluating how the institute will work with its product development partners, but that it is anticipated that the new institute will affect relationships with a "small subset of partners in specific disease research areas."

Lawrence Corey, president and director emeritus of the Fred Hutchinson Cancer Research Center in Seattle and a principal investigator of the HIV Vaccine Trials Network, says he had little knowledge about the concept or genesis of the institute. "But new research into these problems are always welcome and helpful."

Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases (NIAID), the largest public funder of basic research of infectious diseases, agreed. "NIAID puts a substantial effort into TB, malaria, and diarrheal diseases, and we will likely develop collaborative and synergistic relationships with them," he says. "I'm looking forward to seeing how this thing evolves." —*Mary Rushton*

A Virus and a Vector, Evolving

A decade and a half ago a team of researchers came together in Portland to pursue the use of cytomegalovirus as a potential vector for vaccines. Its future will be determined by upcoming human clinical trials.

By Michael Dumiak

Oregon Health & Science University (OHSU) researchers in the state's pine-forested primate research center are preparing to do new things with something very old. Teams there are bolstering techniques and manufacturing practices as they ready an HIV vaccine candidate for safety studies in humans. The candidate employs cytomegalovirus (CMV) as the vaccine vector, a virus with a long history.

Probing further, OHSU researchers are manipulating CMV vectors in the hope of uncovering a tool for tailoring or "programming" different kinds of immune responses. Working with the large non-human primate (NHP) research program there, researchers hope to use CMV to create a "platform" approach to vaccine development, where a single vector is used for candidates against a variety of maladies. This work has gained the notice of high-flying venture capitalists. A US$150 million venture called Vir Biotechnologies launched earlier this year to support and grow the effort. Now it remains to be seen if the CMV candidate generating so much promising data in monkeys will deliver similar results in humans.

Something old, something new

The cytomegalovirus family tree stretches to the Triassic: the period of early amphibians and ferns. The virus has since undergone 200 million to 240 million years of Darwinian selection, making it very good at what it does. CMV infects 50 to 80 percent of Americans, most of whom show no symptoms. It is widespread in the developing world as well. Like other herpes viruses, CMV establishes lifelong latency in its host after infection.

Its persistent and widespread nature is part of what makes CMV an interesting vaccine vector. OHSU researchers are modifying the viral genome and hijacking it to carry HIV antigens, hoping CMV's persistence might also mean lifelong protection against HIV. The vector's other advantages include its low pathogenicity and efficient ability to reinfect, which should mean a CMV-based vaccine would be effective even if someone had already developed immunity to the virus through natural infection. Researchers also favor its large genome, which offers great potential for manipulation—it can express more than 200 proteins.

Louis Picker, associate director of OHSU's Vaccine and Gene Therapy Institute, is a familiar name in the HIV vaccine research field. Jay Nelson, founder and director of the Institute, and Klaus Frueh, an immunologist-turned-virologist who is a senior scientist there, are also integral parts of the operation. Each have their own labs in Portland. All came to the Pacific Northwest by following CMV as a beacon. Nelson was already established as a CMV expert by 1992, coming to OHSU as a molecular virologist interested in the pathogenesis of the virus. The operation then really began to develop in the late 90's and early aughts.

Frueh had been working first at The Scripps Research Institute in La Jolla and then for Johnson & Johnson (J&J), where he was director of an antiviral pharmaceutical research program. He was, at first, interested in studying how viruses evade detection from $CD8^+$ T cells. Peter Ghazal, a molecular geneticist and former trainee of Jay Nelson's, was working then with a viral protein from herpes simplex. Ghazal was the one who brought Frueh's attention to a growing body of work about CMV. Frueh then began focusing on how CMV counteracts $CD8^+$ T cells. Nelson recruited him to Portland in 2000.

By then Picker was already there, coming from Dallas where in 1999 he'd been working as a pathologist monitoring immune responses in specimens from AIDS patients at the University of Texas Southwestern Medical Center. Picker first directly encountered HIV as a resident at Boston's Beth Israel in early 1983, where he was performing postmortems on patients dying of a mysterious virus prompting unusual, awful symptoms and devastating immune system collapse. What would come to be called AIDS continued to affect Picker personally. As the southern California native continued in his medical studies at the University of California, San Francisco, he lost classmates and acquaintances. He published his first clinical pathologic paper on HIV in 1985 and studied human T cell biology for the next decade. He started working on HIV from that point on.

While in Dallas, Picker became drawn to CMV and its potential in vaccine development. It stood out to him because the virus is largely benign and because it induces very strong lifelong immune responses. In long-term, healthy carriers of CMV, upwards of 10 percent of T cells that distinguish self from non-self are devoted specifically to CMV proteins, says Peter Barry, director of the Center for Comparative Medicine at the University of California, Davis. Barry's lab also works with CMV and collaborates with the OHSU researchers.

Picker wanted to act against HIV, and Jay Nelson, CMV expert, already headed the Vaccine Institute. It was a perfect match. Picker, Frueh, and Nelson began collaborating on antigen design. Frueh and Nelson grew experimental batches of CMV vector candidates, while Picker designed and conducted trials with the center's rhesus macaque population. The continuing experiments built up a collection of data illustrating how CMV induces immune responses in monkeys and how these immune responses affect SIV.

In 2009 Picker and colleagues showed that a rhesus cytomegalovirus (rhCMV) vector expressing the simian immunodeficiency virus (SIV) proteins Gag, Rev/Nef/Tat, and Env primed and maintained effective SIV-specific immune responses in 12 vaccinated rhesus macaques challenged with the highly pathenogenic SIVmac239 strain (*Nat. Med.* **15**, 293, 2009). While all of the control group of 16 animals became progressively infected with SIV after repeated low-dose challenges, four of the vaccinated macaques never showed sustained SIV infection. It turned out they completely controlled it.

This suggested that the CMV-based vaccine immediately and completely controlled viral infection, with experiments later showing that this control is followed by clearance of the infection. Results from a study published the next year were the first to show that downregulation of major histocompatibility complex (MHC)-1, a set of cell surface proteins that tell an immune cell about foreign molecules, enabled CMV superinfection. This suggested that widespread pre-existing immunity to CMV would not hamper its use as a vector (*Science* **328**, 102, 2010 and see *CMV Superinfection No Longer Shrouded in Mystery*, *IAVI Report*, Vol. 14, Issue 2, p. 17).

In 2011, researchers showed that the rhCMV vector could produce a 'functional cure' of SIV-infected macaques, likely due to a memory T-cell response (*Nature* **473**, 523, 2011). A follow-up study from Picker's group showed the experimental CMV vaccine also led to undetectable SIV levels in about 50 percent of macaques challenged vaginally and intravenously, as well as intrarectally (*Nature* **502**, 100, 2013). This indicated that the vaccine-induced immune responses can control viral load in the blood as well as the lymphoid tissues where SIV and HIV establish infection, Picker says. Most importantly the study showed clearance of the SIV infection in protected monkeys over time. This was a startling and highly publicized finding, one still generating new experiments. At the most recent Conference on Retroviruses and Opportunistic Infections (CROI) in Seattle, Picker outlined how his group's current efforts to determine whether the SIV reservoir is progressively eliminated by vaccine-induced SIV-specific immune responses, or whether these immune responses initially limit the formation of the viral reservoir to such an extent that it dwindles to nothing over time (see *Rallying CROI*, *IAVI Report*, Vol. 21, Issue 1, p.13). Experiments so far indicate that the rhCMV immunization is

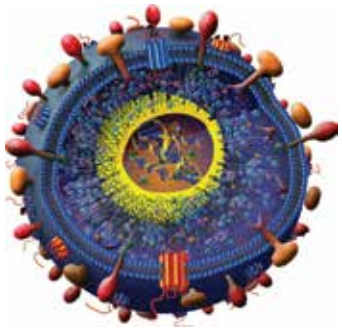
The birth of a vector

Cytomegalovirus (CMV) may have a pedigree stretching back beyond the pterodactyl, but the roots for using CMV as a vector lay in a technique developed in Germany in the 1990s by Ulrich Koszinowski and Martin Messerle.

Messerle and Koszinowski developed a strategy for the cloning and mutagenesis (or recoding of the genetic information of an organism) of an infectious herpesvirus genome. After modification, it is maintained as a bacterial artificial chromosome, which is a DNA construct that can replicate when inserted into bacteria. Messerle and Koszinowski then showed that they could take a mouse CMV genome and maintain it as a 230 base-pair bacterial artificial chromosome when inserted into *E. coli* (*Proc. Natl. Acad. Sci.* **94**, 14759, 1997). Doing so allowed scientists to modify the genome—mutate, insert, or delete pieces of its genetic code—more easily than before. —*MD*

limiting the formation of the viral reservoir, a finding that could have a bearing on research into an eventual HIV cure.

Even now though Picker cautions that a cure strategy is on a much slower track than prophylactic CMV-based vaccines. "An HIV cure is sort of a separate issue. We're asking whether it will work for that. It's a very different kind of application than a prophylactic vaccine, even though the virus is the same. It's just like Hepatitis B. We have a prophylactic vaccine for Hepatitis B. We all take it. It works very well. But we don't have a vaccine that gets rid of Hepatitis B in people that are chronically infected. Therapeutic and prophylactic are two very different things," he says. "We're way more advanced in the prophylactic vaccine."



Modeling CMV. This image illustrates the structure of a cytomegalovirus particle, showing viral glycoproteins embedded in the envelope lipid bilayer, tegument proteins, the capsid structure, and viral DNA genome. Researchers at OHSU are working with CMV as a vector for an experimental HIV vaccine. Image courtesy of Andrew Townsend and Klaus Frueh, OHSU.

Programming an immune response

All the key data on rhCMV as a vaccine vector so far come from Picker's OHSU team, as they are the only ones to publish on it so far, Barry says. OHSU was uniquely positioned to do this work. Researchers there can draw on the resources of the primate center, as well as the scientists with immunology and virology expertise in CMV.

This expertise was assembled step by step, but the run of successful experiments started with a stroke of luck, Picker says. The rhCMV vector that the Oregon researchers began experimenting with has a genetic configuration unlike any other CMV vector. It contains specific gene deletions in discontiguous places that turn out to be required for inducing the specific immune responses required for protection. "If we'd started out with a different CMV variant, we might not have seen this," Picker says. He has five papers lined up on his desk detailing this work, but the ongoing effort to translate the monkey work into human trials is taking precedence these days.

"When we started vaccinating monkeys and ultimately challenging them, we saw a $CD8^+$ T-cell response to SIV," Picker explains. "We had no inkling that there was going to be anything unusual. We saw this efficacy, and to try and figure out correlates for this efficacy we started doing detailed analysis of the immune response." The $CD8^+$ T-cell response to SIV is well characterized following vaccination and natural infection. There are canonical epitopes in this response: a monkey that is said to be Mamu-A01+, which is an analog for the human coding complex for MHC proteins, will typically respond to a certain peptide (*Immunogenetics* **38**, 141, 1993; *Virology* **77**, 9029, 2003).

But when the group vaccinated with a CMV vector, though, they didn't see this kind of response to the canonical epitopes (*Science* **340**, 6135, 2013). "That was an important clue telling us that we should look at the epitopes and what was going on there, because maybe that was important," Picker recalls. The Oregon researchers suspected the control they observed in the vaccinated macaques came from a more promiscuous immune response involving multiple epitopes.

So Frueh and Nelson continued to construct vectors, and Picker tested them. The results created an understanding of the gene coding in CMV that elicits both unconventional and conventional responses. Picker has said publicly in meetings that the unconventional responses are what seem to be required for efficacy. Both Frueh and Picker were recently in the Netherlands at a CMV workshop discussing ongoing research. It appears they are on the cusp of characterizing which viral genes are required to get specific vaccine-induced immune responses. This boosts CMV's prospects not just for HIV but as a potential vaccine platform.

The group is now working to show that genetically altering CMV can actually "program" highly diverse $CD8^+$ T-cell responses that differ in their epitope targeting. "The issue is understanding how that works and understanding not only how those responses are generated by CMV, but also what they are good for," Picker explains. "At this point, we know that they're good for a prophylactic SIV vaccine and, presumably, an HIV vaccine. And in data that we're going to publish hopefully soon, it seems to work for tuberculosis as well." The unconventional responses elicited by their CMV vectors are dependent on the deletion of 157.5/.4 genes (*Science* **351**, 714, 2016), as well as other genes about which the group has not yet published.

Ubiquitous but unique

While the momentum in HIV vaccine research over the last decade has been much on the side of designing an antigen that would prompt or "coach" the body to start producing antibodies against the disease, specifically antibodies that neutralize a broad spectrum of HIV strains, a CMV-based vaccine would work a little differently. It is not intended to induce antibodies, and in its current configuration, it doesn't. It would instead rely on the body's ability to mount a strong T-cell response to CMV. The persistence of the response allows the T cells that it elicits to avoid going into a resting state, so they remain ready to manifest antiviral activity. Picker

describes it as the 'early intercept' hypothesis. "If you meet it at the beachhead, so to speak, it never has the chance to use its programmed host evasion mechanisms, and therefore it is vulnerable."

But some of CMV's advantages could also prove to be concerns: a persistent vector that causes harm would be problematic. While CMV is widespread and largely benign, it can be dangerous in pregnant women and associated with accelerated senescence of the immune system (*Virus Res*. **157**, 175-9, 2011). CMV is also linked by some researchers to poor outcomes in elderly people. These effects seem linked to the persistent immune stimulus caused by the virus's enduring potency.

The Oregon researchers are at pains to say safety is the priority. "We're all very aware of that literature. The way to prevent that is basically to limit the ability of CMV to reactivate and disseminate, and that's why our clinical vectors will be attenuated. All of this literature really depends on the virus being able to reactivate in old people and disseminate, and our vectors won't be able to do that," Frueh says. "The literature on involvement of CMV in immunosenescence is highly controversial. We also need to recognize that basically CMV is part of our immune system. It has been for a long time."

Peter Hunt, who researches the inflammatory consequences of HIV infection from his post as associate professor of medicine at the University of California, San Francisco, also thinks the immunosenescence problem is overstated. "While the dogma that 'CMV accelerates aging' has been in the aging literature for some time, this has been widely misinterpreted and paints too broad a brush," he says. "I have very few concerns about Louis's fibroblast-adapted vector," Hunt adds, referring to Picker and the OHSU vaccine candidate. "His data are some of the most exciting preventative vaccine data to emerge from non-human primate models of HIV infection."

Which may be why the CMV program has drawn the attention of venture capitalists. At the start of this year, a company calling itself Vir Biotechnology made its debut in San Francisco with the backing of a top-flight venture capital firm run by biotechnology impresario Robert Nelsen, as well as funding from the Bill & Melinda Gates Foundation. Set to run the firm is George Scangos, the former chief executive of Biogen, a high-flying Boston biotech founded by Nobel Prize winners. Vir has grand plans. At the heart of the company is CMV.

By 2010, Frueh says, about the time the impressive data was emerging from their CMV vector experiments, the group—Nelson, Picker, Ulrich Koszinowski, and Frueh—began to talk about starting a company to spin off their CMV vector. The research effort was growing larger and it became clear that successful results might lead to a marketable product. In the fall of that year they launched a spinoff called TomegaVax. It fell to Frueh to guide it. He's the one who'd previously worked in industry. "I did have that, though I have no experience in building a startup company. Neither did any of the other founders. We were like the blind leading the blind in some ways," Frueh says. What he had done as part of his job at J&J, though, was to evaluate the stream of biotech interests that approached the company in search of an industry partner: a deal. The experience was quite valuable down the road as Frueh began striding the circuit with TomegaVax, looking for backers.

There are compelling reasons to start a spinoff. One is that as an academic the university owns what you do, for better or worse. In the US companies are also eligible for small business grants. In 2014 TomegaVax landed a $225,000 federal small business research and development grant to pursue a CMV-based vaccine against human papillomavirus, which is a prerequisite infection for the development of cervical cancer.

About this time the Gates Foundation and the US National Institutes of Health were also paying close attention to what was happening at the OHSU primate center. By 2014 Picker's lab received $25 million from the Gates Foundation; last year the National Institutes of Health provided $14 million.

Once OHSU's results began suggesting that by modifying cytomegaloviral determinants that control unconventional T-cell priming it is possible to uniquely tailor the $CD8^+$ T-cell response for each individual disease target, it was clear they were outgrowing TomegaVax's capabilities. "It's not something that we anticipated when we started, that we would be able to do this. We're rewriting textbook immunology," Frueh says. "We were actually asked by the Gates Foundation to find a corporate partner." This is a measure of success, but also a challenge. TomegaVax had set up shop in a Portland biotech hub in a warehouse district along the Willamette River. "The problem there was that everyone loved the technology. It was clear that this was something new. But we didn't have experienced management," Frueh says.

Last September, though, Bob Nelsen came calling. Nelsen, a co-founder and managing director at the venture capital firm ARCH Venture Partners, is based in Seattle and is known at

Forbes as 'Biotech's Top Venture Capitalist.' Nelsen had a vision for CMV. "It's a much bigger vision than we had for the company," Frueh says. "He was really saying, 'Let's build a big startup right out of the gate that has the capability of trying out different platforms and has enough money to do multiple clinical trials.'"

ARCH Venture brought $150 million to the launch of Vir Biotechnologies, with the Bill & Melinda Gates Foundation also contributing as a lead investor. Other funding, according to Vir's January launch announcement, will come from sovereign wealth funds, public mutual funds, philanthropists, and family offices. "Vir seeks to take a new approach, using breakthroughs in immune programming to manipulate pathogen-host interactions. The company will take a multi-program, multi-platform approach to applying these breakthroughs, guided by rigorous science and driven by medical need," the company says on its website. It is adopting a broad technological portfolio, including everything about CMV that was obtained with TomegaVax. Though it's still early days, the roles at Vir Biotechnology are pretty clear: Scangos is chief executive, Picker is scientific advisor, and Frueh is a director.

Inflection points

With the launch of Vir Biotechnologies the Oregon team has reached an inflection point: having secured resources, it is now all about translating the promising data they've collected in monkeys to humans.

In a cramped office off a hospital hallway at OHSU's sprawling teaching hospital in Portland, Marcel Curlin, a young, bushy-haired researcher and a new member of the team with a background in HIV clinical trial research in Bangkok, is leading the effort there to screen potential volunteers for the vector's Phase I safety trials. Curlin expects to screen 400 Portland-area residents to make sure they already carry CMV (and that their partners do as well) and that they are not pregnant or carry other risk factors. The goal is to enroll 75 volunteers for a Phase I trial. The start date, though, is a little fuzzy. There have been challenges along the way involving the CMV vector itself.

One is achieving the right level of attenuation to limit the ability of CMV to reactivate and disseminate, in other words to ensure its safety. The team developed a "safety valve" for the vector to accomplish this by deleting a gene that is essential for virus dissemination. Deleting this gene eliminates safety concerns, but the key is to balance safety with efficacy—an over-attenuated vector may render it less able to induce the types of immune responses that were protective in animal studies.

Another delay is because of the findings on immune response programming. Programming could wind up as a stellar advantage for building a vaccine platform, the very thing that interests Vir in CMV. The researchers don't want to use vectors in humans that have different modifications compared to the original monkey work, so clinical vectors had to be re-designed according to the most recent results. Researchers found another essential change last year that had to be added to the clinical vector.

All told, these efforts have caused the timeline for human trials to slip from an expected start this summer. Picker now says the earliest that trials will get underway is the end of 2018 and that will probably move into early 2019.

"We're rushing to finish up this characterization and to get the correct human vectors. We only figured out in the last year how to make them and they have to get to manufacturing standard," Picker says. "We're running hard to try and do that. Obviously, the monkey model is great, but," he trails off. If CMV's promise doesn't translate to humans, it would be a huge setback. If it works similarly, ARCH estimates it may deliver $500 million to the overall CMV project.

Frueh has emerged as the point person in solving some of the manufacturing issues involved in making a vaccine a real possibility. "I'm actually not a trained virologist," he says. "I'm now the virologist in this program." Yet Frueh is relaxed in his corner office, looking over the primate center's central services complex, a midcentury mod-style building. The primate houses are just beyond, where hundreds of monkeys are either leaping around in cages or in a big open oval, socializing.

Although Picker's primary interest is the basic science, he is finding the translation of the CMV work to humans an interesting (and necessary) challenge. "The reason I haven't burnt out yet is that this isn't just development of a vaccine where you completely understand how it works and the energy is in making it manufacturable," he says. "To do this translation you have to understand the basic science. It's interesting and different and weird immunology. It creates problems—you stretch your colleagues' credulity. But when you come down with solid findings, it's fun." ■

Michael Dumiak reports on global science, public health and technology and is based in Berlin.

Ready or Not, *Here it Comes*

Is the world ill equipped to handle infectious disease outbreaks? In his latest book, Michael Osterholm says yes, and explains why and what the world needs to do about it.

By Kristen Jill Kresge

In a 2015 TED Talk, Bill Gates said that "If anything kills over 10 million people in the next few decades, it's most likely to be a highly infectious virus rather than a war. Not missiles, but microbes…we're not ready for the next epidemic."

In *Deadliest Enemy, Our War Against Killer Germs*, Michael Osterholm, founding director of the Center for Infectious Disease Research and Policy and regents professor at the University of Minnesota, and documentary filmmaker and author Mark Olshaker tackle how the global public health community needs to prepare for epidemics in the 21st century. They outline a nine-point Crisis Agenda to address the major infectious disease threats facing the world today from A to Z, or rather from AIDS to Zika.

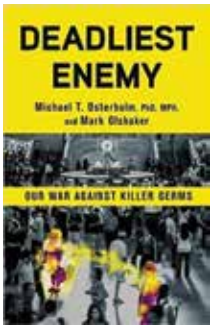
The book opens with a chilling account of what it was like to be sitting around a table in the Director's Conference Room at what is today the US Centers for Disease Control and Prevention (CDC) 36 years ago this month. It was June of 1981 and Osterholm and others were discussing two mysterious clusters of *Pneumocystis carinni* pneumonia (PCP) among otherwise young, healthy gay men in Los Angeles and New York City.

"None of us around the table that day in Atlanta realized that we were bearing witness to an epochal moment in history: the world's transition into the era of AIDS," Osterholm writes in the book.

James Curran, under whose leadership a task force was set up to explore this medical mystery, invited the 28-year-old Osterholm into the room that day. He was at the CDC for another purpose entirely: toxic shock syndrome (TSS). Following an outbreak of TSS in Osterholm's home state of Wisconsin, he became involved in a different medical detective story—what was causing TSS and how could it be prevented? Curran's career, like many others working in public health in the early 1980s, went on to be defined by HIV/AIDS. While Osterholm did not work solely on the virus throughout his career, it affected him both professionally and personally. In 1985 his aunt, a nun and teacher in San Francisco, died an agonizing death from PCP after receiving an HIV-tainted blood transfusion following a broken hip.

"AIDS can serve as a dire warning about the possible: a black swan of an infectious disease that seemingly came out of nowhere, unleashing unimagined suffering on an unsuspecting world," Osterholm and Olshaker say.

Other infectious disease threats are well known, such as influenza. The authors provide a detailed and terrifying description of the death and destruction of pandemic influenza. Some estimates suggest the death toll from the 1918 flu pandemic, often inaccurately referred to as the Spanish flu, was close to 100 million. That is far greater than all the civilian or military deaths due to World War I. "In sheer numbers of human beings killed, the 1918 flu was the deadliest pandemic killer of all time. More people died in a six-month period … than have died from AIDS in the roughly thirty-five years since that virus was identified in the human population," they write. Yet Osterholm and Olshaker estimate that globally US$35 million to $45 million is spent annually on the research and development of more effective and longer lasting flu vaccines, compared to an annual invest-



DEADLIEST ENEMY
Our War Against Killer Germs
By Michael T. Osterhelm, PhD, MPH, and Mark Olshaker
352 pages. Little, Brown and Company.

ment of $1 billion on HIV vaccine research. "Imagine what we could do if research on a game-changing flu vaccine was funded at a similar level to HIV and done in a coordinated and collaborative manner."

And the worst part is that unlike other pathogens, including Ebola, the severe acute respiratory syndrome (SARS) virus, or the Middle East respiratory syndrome (MERS) virus, which most likely will manifest as regional epidemics, epidemiologists know that pandemic influenza *will* strike again. "We don't know which, of all the influenza strains we're watching, will emerge as a pandemic one, or whether it will be something we haven't seen before. What we do know is that when it happens, it will spread before we realize what is happening. And unless we are prepared, it would be like trying to contain the wind," they write. Like I said, terrifying. Even a moderately severe flu outbreak would have dire consequences on global trade and the already taxed healthcare systems of developing countries, similar to but likely much worse than what was seen during the 2014-2015 Ebola outbreak in West Africa.

So why aren't we more prepared for pandemic flu? Well there are probably a multitude of reasons, both financial and scientific, but Osterholm suggests that the public as a whole isn't as concerned about it as other viral threats because they are driven largely by emotion, not reason. There was a great deal of panic across continents during the latest Ebola outbreak, and media coverage was saturated with stories about the link between Zika and microcephaly in newborn babies, but there aren't nearly as many people worried about pandemic flu. "Public health science is based on statistics and probabilities. But we as a population don't think in those terms," the authors suggest. "Rather we think emotionally about things like disease and death." This is what allows us to be afraid of Ebola, but have little concern about antimicrobial resistance that threatens to leave the arsenal of currently available antibiotics largely moot.

In many ways, the world today provides what the authors refer to as a hyper-mixing vessel for pandemic pathogens. Livestock production, which has expanded to support a growing human population, is helping fuel the spread of viruses from animals to humans. As Osterholm pointed out in a recent op-ed article in *The New York Times*, the earth is now populated by 7.4 billion people, 20 billion chickens, and 400 million pigs. Trade and air travel have made the world more interconnected than ever before. And climate change is also aiding the spread of disease, with more and more places on the globe suffering from mosquito-spread viruses, including Zika and malaria.

The best way, the authors contend, to be ready to face these growing threats is to develop and deploy vaccines against the top pathogens. This of course is the goal of the newly formed Coalition for Epidemic Preparedness Innovations (CEPI; see *A Crisis Gives You Wings* and *An Interview with Richard Hatchett*, *IAVI Report*, Vol. 21, Issue 1). Osterholm points out that the role of CEPI, as well as that played by foundations such as the Bill & Melinda Gates Foundation and The Wellcome Trust, are critically important in helping to fill gaps in vaccine discovery. These gaps exist in part because the business model for vaccine development has changed. In 2014, the authors say that the five top drugs generated more than $49 billion in sales for the pharmaceutical companies that manufacture and market them. By contrast, the top five vaccine manufacturers in the world had combined sales of only $23.4 billion that same year. Vaccines are not the money makers they once were and the cost to develop and manufacture them is high. And Osterholm argues that governments haven't stepped up as much as they should, leaving some of the burden of vaccine development to public-private partnerships. This is why he suggests a product-development partnership (PDP) akin to the International AIDS Vaccine Initiative (IAVI) be formed to address flu vaccines.

Osterholm, who is involved in CEPI, sees that model as the best chance for creating a "viable and dependable" pipeline of vaccines for emerging pathogens with pandemic potential. "We should all pay close attention to CEPI's progress," he writes. "Our lives could one day depend on it."

I spoke with Osterholm in May about his book. An edited version of our conversation follows.

In the beginning of the book, you describe what it was like to attend a meeting at the CDC to discuss a new infection occurring among small pockets of gay men, which of course was later identified as HIV. How did that experience shape your career?

You have to put meetings like this into two buckets of perspective. One is what I was thinking about at that time in my career. And second of all, how do you look at it years later in terms of what was happening? You know that some events are history-defining as soon as they happen, like the morning of 9/11. You only really come to understand the importance and significance of other events in history with time.

That first meeting was very significant for me as it came early in my career, and as I said in the book, I felt kind of like I was beamed up to the "mother ship" of public health. But it was only with time that I understood the significance of having been able to

participate in that, and what a special opportunity it was for me to be there. Of course the close relationships that I developed over the years with the CDC professionals working on HIV were also incredibly valuable. Most notably was my relationship with Jim [Curran], who, as I said in the book, was one of the real heroes of the work with HIV, in my opinion. He really did so much to help define the epidemiology in the early days of HIV.

Did you ever consider making HIV/AIDS the focal point of your career?

If you ever worked on HIV in the early days you never did stop working on it, no matter what else you did. In a sense it created a type of presence in your public health soul that you just never lost.

I have remained involved with HIV because even today we have issues with what you would call gay bathhouses reopening in the Twin Cities right now. And so I still find myself, even after all these years, involved with the virus. I also worked for many years to normalize HIV testing so that we could, in fact, effectively address care and treatment in ways that we needed to. For so long there was so much stigma associated with HIV/AIDS.

Back in those early days—1983 to 1985—I worked to make HIV reportable in Minnesota; in fact we were the first government body in the world to make it reportable. It was not about some kind of punishment, it was to help inform some individuals that they were potentially exposed. And when treatment became available, we quickly made certain that we would get these people into the appropriate settings for the latest antiretroviral treatment. And as we all know, the success of antiretroviral therapy in the United States has been nothing short of a miracle. It's been remarkable what has happened.

In a few places in the book, you describe the now-infamous statement made by US Health and Human Services Secretary Margaret Heckler at an April 1984 press conference when she said that an AIDS vaccine would hopefully be ready for testing within two years. You called this "wildly unrealistic." Curran agreed, saying, "The honest question would be, not when there would be a vaccine, but if there would be a vaccine." You have said you didn't believe we would see an effective HIV vaccine in your lifetime. Do you still feel that way or has your thinking changed?

I think that at the time there were two reasons I felt that way. The most important reason was that I did not want us to take our eye off the ball on prevention. What I was really concerned about was that people would say, "We have a vaccine coming shortly; we don't need to worry about primary prevention," which in the end still has been for all these years our most important weapon, in a sense, against this virus. Therapy truly has played a key role in prevention too. That was really the thrust of it. It was not to project some dire kind of crisis mindset, but it was more to make sure that we didn't let people think they didn't need to worry about all this because a vaccine is coming.

The second reason for saying that was just the science. I was sitting there trying to honestly and objectively understand the biology of how we were going to intercept this retrovirus from introduction into the body before it actually creates an ongoing infection. And for me, that was kind of, "Beam me up, Scotty"-type science.

Now, I'm the first to say that with technologic advances this could all change. Maybe today we are doing things that we wouldn't have even considered 30 years ago. I'm still very open to that and that's why I emphasize the need to continue to invest in HIV vaccine research. I would not for a moment suggest retrenching on any of that. We need to continue but I think we just have to be honest about the unique challenges that HIV poses, which the science world has recognized. It's not an inconsistent message to say we have to continue to invest in this even if we don't know if it is possible because a safe and effective vaccine for HIV would be, in a sense, a public health miracle. We can't lose sight of that. We just can't hold off on all of our other efforts with the idea that a vaccine is forthcoming.

You mention in your book how underfunded the efforts are to develop a broader and more effective influenza vaccine, which as you say is the one infectious disease that we can be certain will once again reach pandemic proportions. Do you think HIV vaccine R&D, by contrast, is well funded?

I don't look at it as well funded; I look at it as an appropriately funded effort. I think people want to compare one funding effort versus another, and if you say well funded that tends to allow you to say, "Well, maybe we should move some of the funding over here." When you talk about appropriately funded—in other words, what we must do or should do—then instead of moving funding around, you recognize that we should be increasing funding for the other infectious disease threats. I think people sometimes feel like I'm saying that HIV vaccine research is getting way too much money, but that's not it at all. It just goes to show how underfunded we are for all these other programs. I think that for HIV vaccine research, it's not clear you could really use

much more money effectively, but you sure wouldn't want to have less funding than you have right now.

You outline several factors that are blocking or impeding the development of an improved or as you call it "game-changing" flu vaccine, including the lack of pharmaceutical interest and the failure of governments to really put the money behind this. But yet you quote several people who say this should be a huge priority. Why isn't it?

This is the classic example of what kills us, versus what hurts us, versus what concerns us, versus what scares us. And they all may be very different.

We have had 27 new cases of H7N9 over the last seven days in China. We have had 20-some cases every week over the last month. And normally at this time of year, the seasonal occurrence of H7N9 infection would be waning. It's really concerning. But just like antimicrobial resistance, a vaccine to prevent pandemic influenza is majorly underfunded because both of these are not a crisis that we can yet see or feel. I think that's the mindset we have to get away from. It would be like trying to secure all of your military equipment and all your troops the week after the war is declared. It would never happen like that in the military, yet that's what we do with infectious disease preparedness.

One of the challenges we have is trying to allocate resources in a way that says, "It's not a crisis yet, but this is one that you can't wait until it happens to try to respond." When the next influenza pandemic strain emerges it's going to be way too late to do anything to try to stop its global spread. And our current influenza vaccines will fall so short of what we need in terms of effectiveness and availability. What we're going to go forward with is what we have at that point. If we don't have it by then, we're not going to have it.

And you suggest that with the appropriate level of funding, development of a vaccine to prevent pandemic flu is absolutely possible.

It is. I think there are still many challenges to realizing an effective influenza vaccine because of the science. But I am quite optimistic, based on all we have and what we've done in our own work looking at game-changing flu vaccines, that there are truly those parts of the virus which we can, in fact, use as antigens that could very well produce broad spectrum protection against multiple strains for an extended duration of time.

Imagine taking pandemic flu or for that matter even a lot of seasonal flu off the table as a potential public health crisis.

Are there any shared lessons from research into broadly neutralizing antibodies for HIV that are applicable to the work on flu vaccines?

One of the things that has happened with HIV vaccine research, which I don't think is fully appreciated, is the extent to which it has touched so many areas of immunology and infectious disease research. The basic science research has been absolutely phenomenal. There is no doubt that HIV vaccine research has had a tremendous impact on our understanding and application of human immunology as it pertains to medicine and infectious diseases. So, absolutely, the flu vaccine work has benefitted immensely from that investment in HIV vaccine research.

What do you think is needed to accelerate flu vaccine research?

I believe that the IAVI model should be the primary model for influenza. I think we need that kind of coordinated, collaborative effort—that's as close as we get, in a sense, to a vaccine Manhattan Project. That really is important. I'm a very big fan of the IAVI model. The fact that we don't have an effective HIV vaccine is not a function of a bad model; it's just a function of the tough biologic challenges.

I think the CEPI model, which is really trying to advance vaccine candidates into Phase IIa trials, is really addressing more of a market issue. And even though I'm very involved with CEPI, I've been somewhat critical in that I don't think it's going fast enough. We don't have five more years to get a MERS vaccine. We just had four new MERS cases this morning reported out of Saudi Arabia and they just keep happening and happening. If that virus shows up in East Africa and affects the camel population there, we could need that human vaccine right now.

CEPI is providing a new avenue of funding and it's surely bringing people into the research space that wouldn't otherwise be there, but I see the problem with flu vaccine development as more of a major coordinating issue and that's why I favor the IAVI model. In many ways we are not any better prepared to handle pandemic influenza today, medically, than we were largely in 1918.

What about the Ebola vaccine? The advancement of vaccine candidates during the latest outbreak was lauded as a successful public-private collaboration but is it reproducible?

I think that this public-private partnership is somewhat broken. If we were really looking to pre-

vent Ebola, we would have an African-prepared environment for Ebola. Every healthcare worker in any area of Africa that might experience a spillover would be vaccinated or offered a vaccine—emergency responders, healthcare workers, burial team members, and so on. That's an Ebola-prepared community. We're not there. When Ebola ended in West Africa there was a sigh of relief and it was no longer a priority. Then for two years, nothing much was happening.

These companies have invested hundreds of millions of dollars, with surely some government support and philanthropic support, but they still have put a great deal out there. GSK just walked away from the Ebola vaccine issue because the only pot that they had at the end of the rainbow was a $5 million purchase order for a yet unlicensed vaccine and they had invested hundreds of millions of dollars.

So should there be a PDP for every infectious disease to ensure development of a vaccine?

Yes, in a sense. But the other part of it is that we have to have the push and the pull. Countries need to see that these vaccines are every bit as valuable as any aircraft carrier or missile. And again, we don't wait to purchase ships and missiles until after a war breaks out.

I wrote an op-ed piece in *The New York Times* about a month and a half ago talking about how infectious diseases really are a strategic investment. Our fight against infectious diseases is a national security threat. Imagine if we could take Ebola off the table in Africa, which we're really close to being able to do today. There is Merck's monovalent vaccine and other vaccines out there that look like they could be even much better in terms of likely effective multivalent vaccines.

We all acknowledge that in the past we've stopped Ebola outbreaks without a vaccine, and that's great. But now we know what happens if one of these outbreaks gets out of control, particularly in an urbanized area. We must overwhelm an emerging Ebola outbreak every time we can and a vaccine would be the way to do it. If we had a vaccine then we wouldn't be worried about healthcare workers or burial team members or emergency responders suddenly dying from Ebola, and we'd be much better prepared. This isn't rocket science. It just requires the commitment to doing it.

To accomplish the nine priorities you lay out in the final chapter of your book, what you call the "Battle Plan for Survival," you suggest there should be a major overhaul of the World Health Organization (WHO). Why is this necessary?

The real challenge with the WHO is that it is a system that was put in place at a very different time in world history. It was not made for today's world of public health. There are some really dedicated, wonderful people at the WHO who do work that is just hard to imagine they can do given the constraints they have. If any other organization in the world was led by a board of directors of 194 individuals, there would be chaos. Anybody who took that kind of a scenario into a business class or an MBA program would be laughed out of the room. So part of the challenge is the kind of governance structure that is there. And the financing. The WHO is basically funded on pennies so they have no ability to fund large efforts like something on pandemic influenza. Imagine where we'd be today if the Bill & Melinda Gates Foundation and Wellcome Trust didn't exist. We'd really be in trouble because the world governments haven't stepped up either.

It's not a political issue. It shouldn't even be an economic issue, because you look at the cost savings of investing in these vaccines and it is so clear and compelling. From an economics standpoint, the return on investment is huge.

We all acknowledge, whether it's flu or Ebola or any other disease, that it's not a matter of if it's going to return; it's when, where, and how bad. So it's kind of like preparing for hurricanes. Eventually a hurricane is going to hit us in many of the locations that we routinely have hurricanes, so maybe it happens every 25 years or 50 years, but it is going to happen again. And that's what we have to see with infectious diseases, we need to have that very same kind of mindset and make the same kind of investment.

So the challenge is much greater than the WHO in and of itself. It's about the world's understanding of what it is going to take to provide effective public health in the 21st century. The WHO, as it's now configured and funded, is not it. I'm not being critical of the WHO, they're stuck like this. If I were director general of the WHO, I couldn't do any better than Margaret or anybody else because of the tools that aren't there that are needed.

The world is going to have to figure that out. The UN [United Nations] has to figure it out. And this is where governments like the United States and the EU and Russia and China and everybody else have to come together. There's an old line from a commercial some years ago: "You can pay me now or you'll pay me later." We're not willing to pay now, so we always end up paying later and that's a lot more expensive, and it's unfortunately, a lot more deadly. ■

Upcoming HIV-Related Meetings

JULY 2017

9th IAS Conference on HIV Science (IAS 2017)
July 23-26; Paris, France
More information: www.ias2017.org

AUGUST 2017

2017 Ryan White HIV/AIDS Program Clinical Care Conference
August 21-23; San Antonio, Texas
More information: aidsetc.org/calendar/2017-ryan-white-hivaids-program-clinical-care-conference

SEPTEMBER 2017

2017 United States Conference on AIDS (USCA)
September 7-10; Washington, DC
More information: www.2017usca.org

OCTOBER 2017

10th National Conference of AIDS Society of India (ASICON 2017)
October 6-8; Hyderabad, India
More information: asi-asicon.com/index.php

19th Annual International Meeting of the Institute of Human Virology (IHV 2017)
October 23-26; Baltimore, MD
More information: www.ihv.org/ihvmeeting

DECEMBER 2017

Biomedical HIV Prevention Summit
December 4-5; New Orleans, LA
More information: www.biomedicalhivsummit.org

19th International Conference on AIDS and STIs in Africa (ICASA)
December 4-9; Abidjan, Côte D'Ivoire
More information: icasa2017cotedivoire.org

For a full list of meetings and their descriptions, go to www.iavireport.org/meetings.