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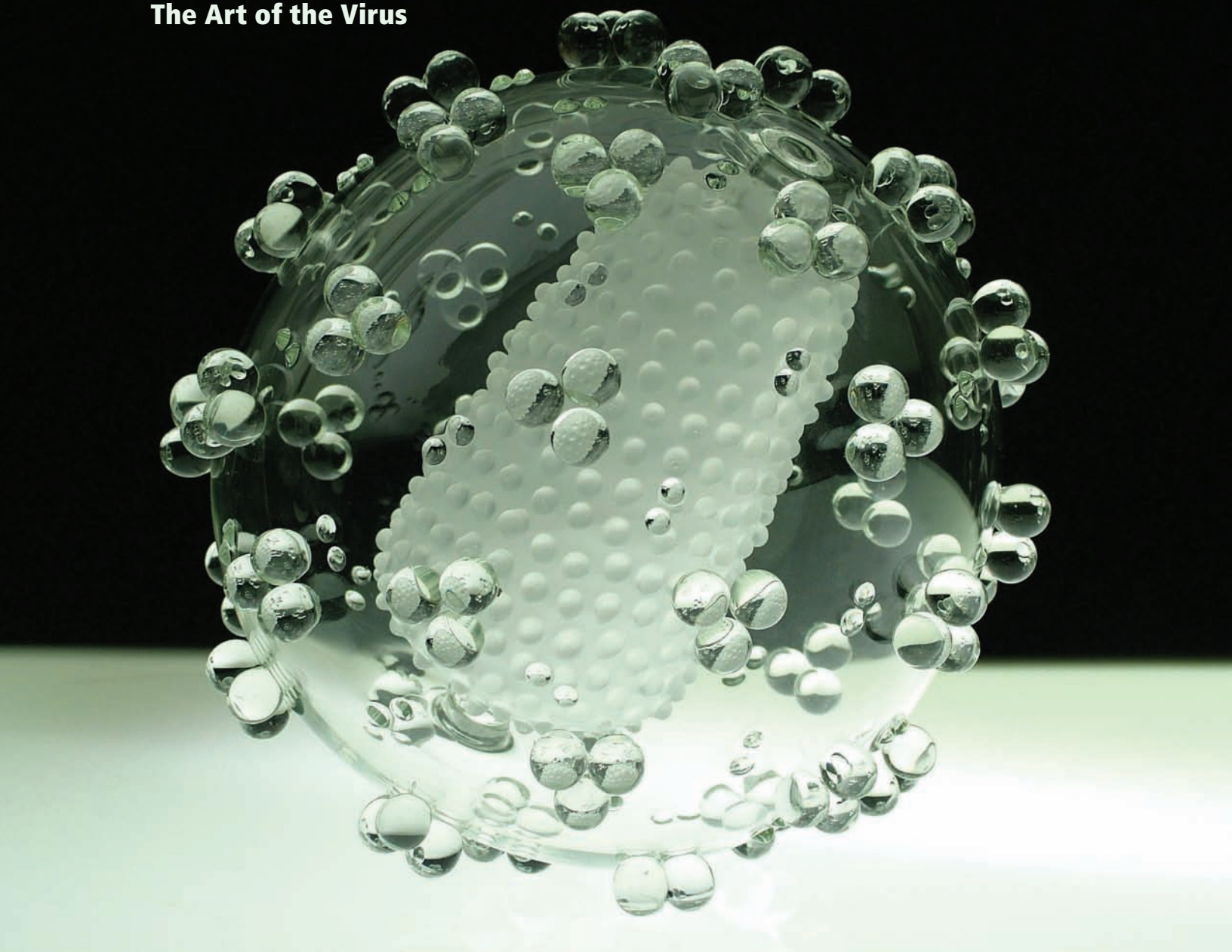
IMMUNOLOGICAL RATIONALE FOR FUTURE VACCINES

PLUS: **Washington, D.C.'s Epidemic**

The Art of the Virus



EDITOR'S LETTER

As 2010 draws to a close, there is more progress to ponder.

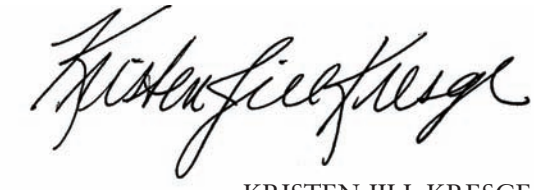
Last year, the field was buoyed by the first trial of a vaccine candidate to show any efficacy in preventing HIV infection. And throughout this year, we chronicled many of the incremental research advances and plans for clinical trials of AIDS vaccine candidates. There was also a burst of good news for other HIV prevention strategies. In July, researchers reported that an antiretroviral-based vaginal microbicide candidate was 39% effective in preventing HIV infection in a cohort of South African women. Then, in November, the first efficacy trial of oral pre-exposure prophylaxis (PrEP) with two antiretrovirals was shown to be 44% effective in protecting against HIV in a cohort of men and transgendered women who have sex with men (see *Vaccine Briefs*, page 18).

While none of these strategies present the perfect prevention option yet, they are important milestones in developing new ways to block HIV transmission, which are still sorely needed despite recent progress in reversing the spread of the virus. Just before World AIDS Day on December 1, the Joint United Nations Programme on HIV/AIDS (UNAIDS) released its updated annual report on the state of the pandemic (see *Vaccine Briefs*, page 19). The latest statistics indicate that HIV incidence has declined by more than 25% over the last decade in 33 countries, 22 of them in sub-Saharan Africa, and that global incidence has dropped 19% from its estimated peak in 1999. This is a tremendous and laudable accomplishment, though UNAIDS warns that it's too soon to declare the battle over.

One place where the battle against HIV/AIDS is still very much a work in progress is Washington, D.C. The US capital has the highest HIV prevalence in the nation, one that rivals many African nations (page 10).

In this issue we also report on the highlights from two recent scientific conferences, the 28th Annual Symposium on Nonhuman Primate Models for AIDS (page 14), and the Keystone Symposium on Immunological Mechanisms of Vaccination (page 4). And to prove that science and beauty can intermingle, we feature an interview with Luke Jerram, a color-blind installation artist who renders viruses, including HIV, in what he calls "glass microbiology"(see cover image and page 8).

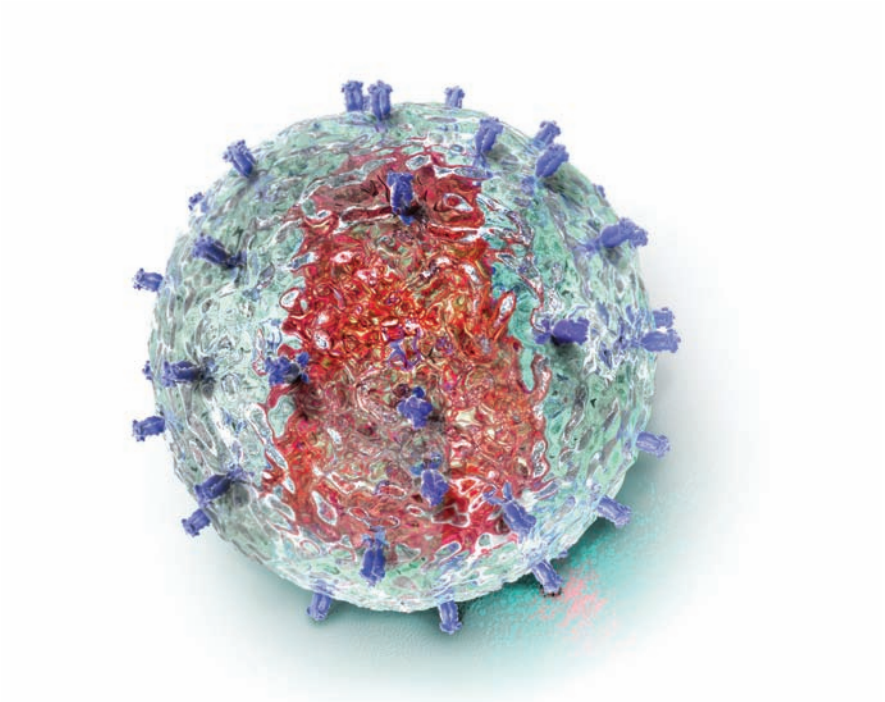
Best wishes for the New Year and here's to having more to ponder in 2011!



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 and operational in 25 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI's financial and in-kind supporters include the Alfred P. Sloan Foundation, the Bill & Melinda Gates Foundation, the Foundation for the National Institutes of Health, The John D. Evans Foundation, The New York Community Trust, the James B. Pendleton Charitable Trust, The Rockefeller Foundation, The Starr Foundation, The William and Flora Hewlett Foundation; the Governments of Canada, Denmark, India, Ireland, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government, the European Union as well as The City of New York, Economic Development Corporation; multilateral organizations such as The World Bank; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, Google Inc., Henry Schein, Inc., Merck & Co., Inc., Pfizer Inc, and Thermo Fisher Scientific Inc.; leading AIDS charities such as Broadway Cares/Equity Fights AIDS and Until There's A Cure Foundation; other private donors such as The Haas Trusts; and many generous individuals from around the world. For more information, see www.iavi.org.

IN THIS ISSUE



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[ON THE COVER]

Glass sculpture of HIV by the British artist Luke Jerram (see Q&A, page 8). The sculpture has a diameter of 22 cm and was made in 2009.

Photograph by Luke Jerram.

An Immunological Rationale FOR VACCINES

A recent meeting brought immunologists and vaccinologists together for the first time to discuss rational vaccine development

By Andreas von Bubnoff

"We really don't know how to make vaccines in a predictable way. It's still a little bit of black magic." That was one of the messages Tachi Yamada, president of the global health program at the Bill & Melinda Gates Foundation, had for the attendees at this year's Keystone Symposium on Immunological Mechanisms of Vaccination, held Oct. 27-Nov. 1 in Seattle, Washington.

Bali Pulendran, one of the conference organizers and a professor of immunology at Emory University, agreed and said that a more rational approach is needed for future vaccine development using insights from immunology. "What's happened in the past is that most vaccines have been made empirically without a real immunological rationale," Pulendran said. "That's worked very well for many vaccines like smallpox, yellow fever, and so on, but increasingly with the more difficult vaccines like HIV, TB, [and] malaria, that's not going to work because the immunological issues are much more complex."

Therefore, one goal of this year's conference was to bring vaccinologists and immunologists together, Pulendran said. To encourage dialogue between the two groups, the organizers intentionally mixed talks on the two research areas in the same sessions. An unusually diverse array of topics were covered at the meeting, including the development and mechanism of action of adjuvants, systems biology approaches to studying vaccination, and the immunological mechanisms that give insights as to why some HIV candidate vaccines might have failed and how they could be improved.

By most accounts, the pairing of vaccinologists and immunologists made for a successful meeting. In his closing remarks, conference co-organizer Rino Rappuoli, a vaccinologist who is head of research at Novartis Vaccines and Diagnostics, said he hopes this won't be the last time the two groups interact. "In order to approach the problems of the future, we need to do better than nature," he said. "So far we managed to do as good as nature. If we want to do better than nature, we need to get together."

Short and sweet

Perhaps a good example of how basic immunology can inform vaccinology was a talk by Surojit Sarkar, an assistant professor of immunology and infectious diseases at Pennsylvania State University. He reported on experiments in mice that suggest that the adenovirus serotype 5 (Ad5)-based vaccine candidate (MRKAd5) developed by Merck may not have induced a protective $CD8^+$ T-cell response

in the Phase IIb STEP trial because the CD8+ T cells it induced were dysfunctional and exhausted.

The conventional wisdom for traditional vaccinology, Sarkar said, is that antigen needs to be expressed continuously for a vaccine to induce a strong and lasting immunological memory. But Sarkar's lab, working with the lab of Vandana Kalia, also at Penn State, found evidence that actually, antigen should only be around for a limited time during the initial few days when the antigen induces naive CD8+ T cells to become effector CD8+ T cells, which can kill the pathogen. But for these effector T cells to then develop into efficient memory T cells, antigen must be absent, he said. Otherwise, the memory CD8+ T cells will be exhausted and dysfunctional. Persistent low levels of antigen, he said, might explain why MRKAd5 did not induce a functionally optimal CD8+ T-cell response. "If you are making a good vaccine, make it short and make it sweet," Sarkar said.

In mice, Sarkar and colleagues measured the quantity and the quality of the CD8+ T-cell response to acute viral infections that are naturally cleared by the immune system, such as infection with the Armstrong strain of lymphocytic choriomeningitis virus (LCMV) and vaccinia virus (VV). Because such infections are naturally cleared, the immune response to them "must be doing something right," Sarkar said. "We take that as a gold standard." The researchers then compared this to the CD8+ T-cell response to Merck's Ad5 vector used in the STEP trial carrying an LCMV antigen.

They found that the quality of the Ad5-induced memory CD8+ T cells was worse than that of the LCMV-induced cells in that they were shorter lived, less likely to produce several different functional anti-viral cytokines, and less able to proliferate and induce protection in response to a secondary pathogen challenge. Their gene expression signature was similar to dysfunctional, exhausted CD8+ T cells observed in chronically infected people. "This signature is enriched only in dysfunctional and exhausted T cells that see longer duration of antigen," Sarkar said. Consistent with this, preliminary analyses of human samples from the STEP trial also show that the CD8+ T cells induced in the vaccinees are not very likely to produce several different cytokines, Sarkar said.

Antigen encoded by the Ad5 vector seemed to be expressed much longer in the Ad5-vaccinated mice than viral antigens are expressed in the LCMV-infected mice. And when Sarkar and colleagues removed the Ad5-induced CD8+ T cells from antigen exposure after one week (by placing them into naive, unvaccinated mice), the cells developed into better quality CD8+ memory T cells. "We think that the antigen persistence in Ad5 is [one of the reasons] the quality of Ad5-induced memory differentiation is impaired," Sarkar said. "You need to give that effector cell a long time of rest so that it can recharge to become a good memory cell."

To develop a vaccine that can induce a better quality CD8+ T-cell response, Sarkar and colleagues are now experimenting in mice with a modified Ad5 vector, the expression of which can be turned off on demand by certain bio-molecular agents that can theoretically be given as a pill, such as the antibiotic Doxycyclin. "If that works, we may try to test it in nonhuman primates," Sarkar said.

Understanding adjuvants

Adjuvants, an important ingredient added to many vaccines to enhance the immune response, were discussed by several speakers at the conference. Although adjuvants are used in many existing vaccines, researchers still don't understand how most of them work. Alum, for example, which consists of insoluble aluminum salts, was discovered about 80 years ago and is used in three quarters of all our childhood vaccines, according to Stephanie Eisenbarth, an instructor at Yale University who recently started her own lab there. However, "we still don't know why it's a good adjuvant," said Philippa Marrack, an immunologist who also studies alum at National Jewish Health, a nonprofit hospital in Denver. Marrack's lab has looked at many of the cell types that are attracted by alum injection to see if they are required for its adjuvant effect. "We have done experiments in which we eliminate each of those cells that are showing up separately and [it] has no effect on the adjuvant activity," Marrack said. Perhaps, she said, alum acts in so many different ways simultaneously that it's hard to understand which one is important.

One hint as to how alum might enhance immune responses comes from a finding by Eisenbarth in collaboration with Richard Flavell, also at Yale University. They found that in mice, the induction of immune responses by alum requires activation of an intracellular sensor called NLRP3, which is part of a so-called inflammasome, a multiprotein complex which activates inflammatory processes after the detection of pathogens or cellular stress (*Nature* **453**, 1122, 2008). However, Marrack said her lab has been unable to confirm these results. While Marrack confirmed that alum

activates NLRP3 in mice, she could not confirm that this activation is required for the induction of immune responses by alum in mice.

Another clue as to how alum might work came in a talk by Ken Ishii, a professor at Osaka University, who suggested that part of the effects of alum might come from the fact that it kills cells, which results in release of DNA. Some of the adjuvant effects could then come from the DNA.

Alum is believed to mostly induce CD4+ T-cell and B-cell immune responses. Hana Golding, a principal investigator at the US Food and Drug Administration (FDA), reported that when it comes to the quality of the humoral immune response to flu vaccine, alum is not the best choice as an adjuvant. Golding said that the oil-in-water adjuvant MF59, not alum, can direct the human antibody response induced by inactivated H5N1 avian flu vaccine to epitope targets in the HA1 part of the viral hemagglutinin protein, which are more important for protection than the epitope targets in the HA2 part (*Sci. Transl. Med.* **2**, 15ra5, 2010). She said that unpublished data suggest that MF59 has a similar effect in people vaccinated with pandemic H1N1 vaccine.

Contrary to the predominant belief that alum primarily induces CD4+ T- and B-cell responses, Marrack reported that, at least in mice, injection of alum together with ovalbumin (OVA; the main protein found in egg white) as an antigen can induce OVA-specific CD8+ memory T cells. "[This] was an unexpected result, an accident actually," Marrack said. These CD8+ memory cells are not very good killers, but when alum is given in combination with monophosphoryl lipid A (MPL), a detoxified form of bacterial lipopolysaccharides (LPS) that is used as an adjuvant in licensed vaccines, the killing capacity of these induced CD8+ T cells can be improved. Further experiments showed that mice vaccinated with flu vaccine containing a small part of an internal protein of the influenza virus were better protected against flu challenge eight weeks later when alum and MPL had been added to the vaccine than with alum addition alone. Because the vaccine in these experiments contains an internal viral protein inaccessible to antibodies, Marrack said this protection is likely due to the CD8+ T-cell response. Therefore, adding alum and MPL to human flu vaccines wouldn't necessarily prevent flu infection, Marrack said, but would make people less sick and therefore less likely to infect others.

Not everyone was convinced, however, that Marrack's finding in mice is relevant to humans. The induction of a CD8+ T-cell response by alum is not found in humans, Rappuoli said, adding that often, T-cell responses are reported from studies in mice without understanding whether the findings are relevant for humans. This is an example of the disconnect that exists between immunology and vaccinology that he hopes the dialogue started at the meeting can solve.

Developing new adjuvants

While alum and MPL are the only two adjuvants that are added to licensed vaccines in the US, Norman Baylor of the FDA said new adjuvants are becoming necessary because as newer vaccines are purified to make them safer and less reactogenic, the natural adjuvants they contain are removed. "The irony is that when we were using whole cell products we had natural adjuvants," Baylor said. "We purified these products and now we need the adjuvants back so we've got to make new adjuvants and then we face potential safety issues all over again."

Steven Reed, director of research and development at the Seattle-based non-profit Infectious Disease Research Institute, reported on the status of the development of a new adjuvant called GLA, a synthetic glycolipid based on MPL. A synthetic compound has the advantage that it can be better purified. "Having something pure allows you to produce your product at a lower cost, keep the dose very low because you are only injecting what's active, and to control your manufacturing process," he said. Reed and colleagues chose to base their synthetic compound on MPL because MPL is already used in licensed vaccines, including a hepatitis B vaccine and the human papillomavirus vaccine Cervarix, both manufactured by GlaxoSmithKline. Reed said MPL has been shown to reduce the number of doses required for the hepatitis B vaccine, and with Cervarix, MPL broadens the immune response to include antibodies against serotypes not included in the vaccine.

Because MPL is a glycolipid and activates the Toll-like receptor (TLR) 4 pathway, Reed and colleagues identified GLA by screening a library of synthetic glycolipids for their ability to activate the TLR4 pathway in cultured human dendritic cells and macrophages, antigen presenting cells that are crucial for generating the humoral and cellular immune response by activating CD4+ and CD8+ T cells. While alum tends to primarily induce humoral immune responses, GLA induces or increases both humoral and cellular immune responses, according to Reed. And in mice and nonhuman primates, GLA is safe and can broaden the immune response to flu strains

not contained in the flu vaccine.

Results from a Phase I clinical trial also suggest that GLA is safe when added to injectable influenza vaccines that contain inactivated flu proteins, said Reed. Another Phase I clinical trial showed "very promising" dose sparing effects, which means that less vaccine may be necessary to achieve the same immune response, Reed said. Next year, Reed and colleagues are planning to test GLA in clinical trials of HIV vaccine candidates.

The systems approach

Systems biology is becoming increasingly important for characterizing the effect of vaccinations on the immune system, and at the conference several speakers described projects using this approach. In an update on his experiments using a systems biology approach to study the immune response to yellow fever and flu vaccines (see *A Systems Approach to Understanding Vaccines, IAVI Report*, July-Aug. 2010), Pulendran showed that microarray data can reveal quite unexpected functional links between the immune response and other biological processes.

Pulendran's previous study of the immune response to the yellow fever vaccine found that a gene called *EIF2AK4* or *GCN2* was correlated with the magnitude of the later adaptive $CD8^+$ T-cell response to the vaccine (*Nat. Immunol.* **10**, 116, 2009). Pulendran and colleagues have now shown that *GCN2* knockout mice had reduced $CD8^+$ T-cell responses to yellow fever vaccine, suggesting this gene was required for the adaptive immune response. While it was known that the protein encoded by *GCN2* is phosphorylated in response to amino acid starvation of cells in response to stress, which results in a shutdown of translation, the link of *GCN2* to the adaptive immune response was not known before, Pulendran said.

Microarray analysis of the response to the flu vaccine revealed another unexpected functional link of a gene to the immune response. The analysis identified a gene, *CaM Kinase 4*, the expression of which just three days after vaccination was negatively correlated with the HA antibody titers four weeks after vaccination. This was confirmed in *CaM Kinase 4* knockout mice, which showed strongly increased antibody titers in response to a flu shot. "[This shows] that by combining rigorous human experiments with mechanistic animal model experiments, you can learn really new biology," Pulendran said.

Ronald Germain, director of the program in systems immunology and infectious disease modeling at the National Institute of Allergy and Infectious Diseases, and colleagues have also been using a systems biology approach to look at the response to flu vaccination. Germain, who is also an associate director at the Center for Human Immunology, Autoimmunity and Inflammation (CHI), which was recently established by the US National Institutes of Health, presented an update on a CHI project that is characterizing, in unprecedented detail, the immune response of 160 people before and after vaccination with the killed version of the seasonal flu virus and the H1N1 swine flu virus (see *A Systems Approach to Understanding Vaccines, IAVI Report,* July-Aug. 2010). The project collects data prior to vaccination to establish baseline measurements and help define the normal so-called "immunome," and post-vaccination data on day one for the innate response, day seven for the adaptive response, and day 70 for the memory response.

Among other things, the analyses so far include measurements of genome-wide gene expression and more than 60 cytokines in blood. Preliminary results from over 60 of the individuals suggest that many gene expression changes occur as early as one day after vaccination, some of which are changes in innate immune response genes. But Germain said one potential concern is that at least some of these early gene expression responses one day after vaccination could simply be due to stress related to the injection rather than the vaccine itself. To address this problem, he plans to measure responses to a mock injection.

Germain also mentioned another challenge with gene expression analyses that was repeatedly discussed at the conference. When researchers use microarrays to measure the expression of thousands of genes in mixed cell populations, the data of gene expression changes might simply reflect changes in the abundance of certain cell types and not cell type specific gene expression changes. "This comes up again and again and again," Germain said.

One way to address this problem is to separate the cell types first and then do gene expression analyses. However, Germain said, this can be expensive if it has to be done for many samples, and the number of available cells can also be limiting for some subjects. Less expensive bioinformatic tools are being used to extract the cell type specific data from microarray measurements, Germain said, but experiments in influenza-infected mice suggest that this approach can miss important gene expression changes that reflect disease state. "Cell sorting may be critical in some cases," Germain said. ■

[IFN EFFECTS]

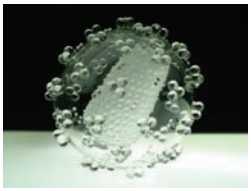
In Seattle, researchers reported that the expression of interferon (IFN)-induced genes can predict vaccine effects. Rafick Sékaly, chief scientific officer and co-director of the Vaccine & Gene Therapy Institute in Florida, in collaboration with Louis Picker, a professor at Oregon Health & Science University, vaccinated rhesus macaques with different live-attenuated vaccines, including simian immunodeficiency virus (SIV) mac239Δnef, and challenged them intravenously a year later with SIVmac251. When the researchers checked gene expression nine days after the initial vaccination they found that the macaques that later turned out to be best protected had a high IFN response shortly after vaccination, but a low IFN response after challenge, whereas macaques that were not protected showed the opposite profile.

IFN-inducible genes can also predict adverse events. I-Ming Wang, an associate scientific director at Merck Research Laboratories, found that genes induced by IFN can to predict adverse events in response to a vaccine. —*AvB*

Photograph by Thes

The Art of the Virus

Luke Jerram turns viruses into sculpture. Why would anyone do this?



Luke Jerram is an artist based in Bristol, UK, who creates glass sculptures of pathogens, including the viruses HIV, coronavirus, smallpox, and swine flu as part of a project he calls "glass microbiology." In 2009, the Mori Museum in Tokyo exhibited his swine flu sculpture in a show called Medicine and Art, along with works by Damien Hirst, Andy Warhol, Marc Quinn, and Leonardo da Vinci. Earlier this year, the Heller gallery in New York City showed some of Jerram's glass virus sculptures, including HIV. Some of the HIV sculptures can be seen now at the Wellcome Collection in London, the Bristol City Museum, and the Corning Museum of Glass in Corning, NY. For more about Jerram's work, see www.lukejerram.com.

What gave you the idea to make glass sculptures of viruses?

I am red-green color blind, and I am interested in how we see and perceive the world and in exploring the edges of perception. I am interested in looking at things that we can't see with our own eyes. When looking through telescopes and microscopes, you start to realize that there is a discrepancy in what you see and the imagery that you see presented through the media and the press. Viruses in the press are often artificially colored using Photoshop and things like that so the public believe that the viruses are these brightly colored things, whereas actually they are transparent and three-dimensional, so that's why I made them transparent and 3-D.

What color do most people think viruses have?

Oh, just reds and yellows, and pinks and purples. Interestingly, an electron microscopic image will be black and white, but then those images sometimes go to organizations like the Science Photo Library in London, where they actually Photoshop them to give them a greater emotional content. Sometimes they are very pretty; sometimes they are decorated like poisonous fungi. Then they sell those images to journalists. Journalists phone up and say, 'I need some healthy looking bacteria,' and they'd say, 'Oh yeah, you need the green and white ones.' When journalists want some dangerous looking bacteria, they would say, 'You need the purple and yellow ones.' It's just very confusing for the public because the public doesn't know whether it's Photoshopped and colored for scientific reasons or for aesthetic reasons, to add emotional content, and you don't know who created them. It's just complicated and confusing, I think.

Why did you choose viruses, and HIV in particular?

Well, viruses are right at the edge of what you can see with an electron microscope. If you look at images of viruses through an electron microscope they are incredibly blurry and out of focus. So when I am speaking with virologists about what the viruses look like, we have to sort of jump between what we see through an electron microscope and what we can understand through models and through looking at diagrams. The sculptures are a combination of electron microscopic imagery and diagrams of viruses.

I have done a number of viruses and I do ones that generally people are aware of and care about.

Would you say the sculptures are beautiful?

Yeah, it's interesting. The objects are very beautiful, but when you realize what they are then they are slightly repellent, so that creates an interesting tension.

How do people respond to them?

They are generally amazed. People suffering from HIV send me stories thanking me for making the sculpture. They are unable to visualize it and now they are able to see the devil, the likely cause of their own death.

How do you make the sculptures and how long does it take to create one?

It takes a number of months. I collect all visualizations of the same virus and then I meet with a virology expert and do some drawings and we talk about what it would actually look like and about different ways to represent different components. Then I send drawings to my glass team and we do prototypes and test those out. I am often coming up with designs that physically won't withstand forces of gravity, so you are dealing with the limitations of glass blowing. Sometimes designs are just too fragile to be made and shipped and transported, so there are lots of complications, but it's a lot of fun.

Are there any issues when you ship sculptures named after viruses?

Yeah, I have to code them. I can't say this is an HIV sample or SARS. I have to give them a code name so that they can go through customs. I had a friend who took a sample on the airplane and we couldn't say, 'You know, I am just carrying my smallpox sample through the airport.' It just wouldn't go down very well.

What do you name them?

I couldn't say that. I could get in trouble.

What are you going to do next?

I have been invited to do a residency at the Museum of Glass in Tacoma, Washington. We may end up making a huge virus sculpture there.

Interview by Andreas von Bubnoff

Why is HIV Ravaging D.C.?

The capital of one of the richest nations in the world is beset by an HIV epidemic that rivals those seen in some developing countries

By Regina McEnery

Twenty-nine years ago, the US Centers for Disease Control and Prevention (CDC) issued a brief report in its Morbidity and Mortality Weekly Report about an unusual cluster of pneumocystis pneumonia infections among five gay, otherwise healthy men from Los Angeles. A month later, 46 more cases were reported in Los Angeles, San Francisco, and New York City.

Today, the majority of new HIV infections in the US still occur in men who have sex with men (MSM)—approximately 77% of all infections among men and slightly more than half of all new infections overall occur in MSM. Although the prevalence rate of HIV/AIDS in the US is high in subgroups such as MSM, the overall HIV prevalence rate among adults is estimated to be less than 1%, according to the Joint United Nations Programme on HIV/AIDS (UNAIDS). As such, UNAIDS considers the US to have a concentrated HIV epidemic, primarily among MSM and injection drug users (IDUs), rather than a generalized epidemic such as South Africa's, where more than 1% of the entire population is infected with HIV and mere geography becomes a risk factor for acquiring the virus.

However, there is now growing evidence that a more generalized HIV epidemic has emerged among heterosexuals in poor, urban neighborhoods stretching across the US from Boston to Seattle. Nowhere is it more evident than in Washington, D.C. The capital of one of the richest countries in the world has an HIV prevalence rate comparable to those in developing countries (*Health Affairs* **28**, 1677, 2009). In 2007, an estimated 3% of the District of Columbia's adult population was infected with HIV, a higher HIV prevalence rate than Rwanda, Angola, and Ethiopia, and just slightly lower than Nigeria and the Democratic Republic of Congo.

Other estimates suggest the HIV prevalence in Washington, D.C. may be even higher. A George Washington University (GWU) study based on data collected from December 2006 to October 2007 for the National HIV Behavioral Surveillance (NHBS)—a community-based study funded by the CDC and the District of Columbia Department of Health—estimates that the HIV prevalence rate in the district among a particular subset of heterosexuals at high risk for HIV infection is as high as 5.2% (*AIDS* **23**, 1277, 2009). The study's authors said this was "the first estimate of HIV and risk behaviors among urban, low income, and African Americans in the nation's capital." The HIV prevalence among women in this study was 6.3%, similar to the prevalence among women in Tanzania (7.0%) and Uganda (7.1%).

One reason D.C.'s HIV prevalence is the highest in the nation is the district's size. The city's population of roughly 590,000 residents is small compared to other urban areas with significant epidemics, such as New York (population 8.3 million) or Los Angeles (3.8 million). But a number of troubling epidemiological, social, and political factors are also to blame. Poor disease reporting, a lack of laws to support syringe-exchange programs for IDUs, and poverty, among other factors, have all contributed to the burgeoning epidemic in the US capital. While fighting the epidemic there is still an uphill battle, the city's public health officials have made some significant strides in recent years to understand the epidemic and to adjust prevention programs accordingly.

The roots of the epidemic

Evidence of a generalized epidemic in D.C.'s poor, urban neighborhoods reflects what seems to be happening nationally within economically disadvantaged neighborhoods. A poster presented by the CDC at the XVIII International AIDS Conference in Vienna in July showed that the HIV prevalence between September 2006 and October 2007 is estimated to be 2.1% among 23 urban poverty areas in the US, including parts of Washington, D.C. that the NHBS now tracks on a regular basis. Poverty areas are defined as those where at least 80% of residents have household incomes below the US poverty level. A family of four is considered to be living below the poverty level if their household income is below US$22,000 in the 48 contiguous states or Washington, D.C., $25,000 in Hawaii, and $27,500 in Alaska. Because the study was designed to study the potential link between poverty and HIV risk, the NHBS sample analyzed by researchers excluded other risk groups such as MSM, IDUs, and commercial sex workers (CSWs) living in the 23 urban areas.

The CDC found that HIV prevalence among individuals who were surveyed in the urban poverty areas was inversely proportional to socioeconomic status—the lower the income level the greater the prevalence of HIV—and that unlike overall HIV prevalence in the US, HIV prevalence in urban poverty areas did not differ significantly by race or ethnicity. "Poverty isn't itself the direct cause of HIV infection," says Jonathan Mermin, director of the CDC's HIV/AIDS prevention program that presented the paper in Vienna. "Most people get HIV from having unprotected sex. But poverty sets up an environment that increases the possibility that someone will be having unprotected sex."

In addition to poverty, several other factors are driving the epidemic in Washington, D.C. One is a scattershot HIV/AIDS surveillance system that until 2006 failed to collect or report data on a timely and consistent basis. Only recently, in 2008, D.C. converted to a confidential name-based reporting system that the CDC has used since the early 1990s, and recommended states and dependent areas begin using in 2005, to collect data on HIV infections and monitor the epidemic more effectively (see *A Static Epidemic*, *IAVI Report*, May-June 2008).

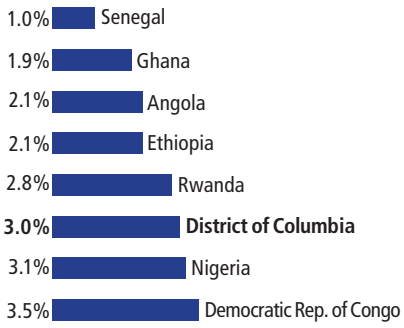
The lag in reporting HIV cases has made it difficult for both public health departments and privately run AIDS agencies to fully appreciate the status of the current epidemic, which in turn has hampered efforts to develop effective responses to control it. DC Appleseed, a non-profit organization based in the US capital, has been issuing annual report cards on the district's response to HIV/AIDS for the past five years and says that the lack of access to timely epidemiological data has delayed the implementation of prevention plans that determine how much money should be spent and where the resources should be allocated. "Accurate and complete HIV surveillance data are essential to plan HIV prevention programs and allocate healthcare resources," DC Appleseed noted in its inaugural report in 2005. "Although the HIV/AIDS Administration (HAA) has collected HIV data for the past three and a half years, it has not yet publicly disseminated a report on HIV data."

The political structure of Washington, D.C., a federal territory that despite being the capital has less legislative representation than the 50 states and falls under the jurisdiction of the US Congress, has also created tensions between local and federal officials that have made it difficult to effectively tap resources that might be used for HIV prevention and care for people with HIV/AIDS living in the city.

Some critics also blame the district's high prevalence rate on federal laws that have barred the public funding of syringe exchange programs, which a number of studies have shown are effective in reducing HIV transmission among IDUs (see *A Static Epidemic*, *IAVI Report*, May-June 2008). Until this year, the US government banned federal funding for programs that provided IDUs access to clean needles because needle exchange programs were viewed as being supportive of illegal drug activity. Like many states and cities, Washington,

[HIV/AIDS PREVALENCE]

Selected Countries and Washington, D.C., 2007



Source: D.C. HIV/AIDS Administration

D.C. had been relying on privately funded needle exchange programs, but the district's effort was only able to reach about a third of the city's IDUs, according to DC Appleseed's 2005 Report. In 2008, D.C. was granted Congressional approval to use district funds for needle exchange programs.

The high HIV prevalence in the district is also being blamed on the absence of a comprehensive counseling and testing policy and weak HIV education programs in the public schools.

Mermin says the social determinants of health—the circumstances in which people are born, grow up, work, and age, and the systems in place to deal with illness—are also drivers of the epidemic. "Syringe services can be effective in reducing the likelihood that people who inject drugs will acquire HIV. Having access to accurate information about HIV transmission can help people avoid acquiring HIV," says Mermin. "But it would also be beneficial for HIV prevention if there were no poverty in these areas. Homelessness, low household income, lack of education, lack of employment are also independently associated with [an] increased risk of HIV."

The US's first National HIV/AIDS Strategy, which was released in July by US President Barack Obama's administration and created to provide clarity and coordination in the prevention and treatment of HIV, seems to reflect Mermin's view (see *Vaccine Briefs*, *IAVI Report*, July-Aug. 2010). The 60-page document noted that it is important to employ a holistic approach to HIV prevention and care that extends beyond risk behaviors of the individual to "contextual factors" such as joblessness, homelessness, and sexual- and drug-use networks. "Although there have been some successful efforts in this regard, such as interventions that examine the link between homelessness and HIV risk behavior, there are too few proven models associated with reducing HIV incidence that have had a community-level impact," according to the report.

Robert Fullilove, a professor of clinical social sciences at Columbia University, has studied the US epidemic in urban communities for more than 20 years. He agrees with Mermin that poverty has driven the epidemic in the US as much as, if not more than, risky behavior. Fullilove says structural factors such as unemployment, incarceration, racism, and neighborhood violence have created an environment that has put people living in these communities at greater risk for HIV. And, he says, black men and women are disproportionately affected by HIV because a higher percentage of blacks live in poverty.

"A number of us have written about this extensively," says Fullilove. "We've felt that people were paying much too much attention to individual risk factors and not enough to the geography of the epidemic, which is mostly urban and mostly confined to poor urban communities of color."

The geographical risk of HIV infection in the US was evident in a 2006 study that set out to determine the risk factors for HIV among blacks in North Carolina by comparing recently HIV-infected individuals with a matched control group of HIV-uninfected individuals. The researchers, including Fullilove, found that while most of the individuals reported either high-risk behaviors such as crack-cocaine use or having sex partners that injected drugs or used crack cocaine, about 30% of the HIV-infected volunteers and 69% of the uninfected volunteers denied any high-risk sexual partners or behavior. Instead, the risk factors for these individuals were lack of a high school education, concerns about not having enough food to feed their family, and having a partner who was not monogamous (*J. Acquir. Immune Defic. Syndr.* **41**, 616, 2006).

D.C. fights back

While the situation in Washington, D.C. is the worst in the nation, the capital city has made some significant strides in recent years. For one, the district has greatly expanded the quality of data about residents who are either at risk of or are already infected with HIV, and its eventual goal is to develop a database that collects and stores information, in real time, from all consenting HIV-infected individuals undergoing care and treatment at major clinics throughout the city.

Shannon Hader, who was appointed director of HAA in 2007 and left in May to join the international development firm Futures Group, says the improved data collection is allowing the district, for the first time, to develop policies that directly address the epidemic.

"For many years the district had been dysfunctional, unable to collect, process, and disseminate data, much less apply a national surveillance system," says Hader, who is widely credited with turning around the failing administration. "If you don't have data you fund programs and prioritize activities based on anecdotes and impressions. Now that we have the data the numbers really speak for themselves. The numbers tell us we have a serious and extensive epidemic in the capital that affects many groups and all modes of transmission. Despite the heavy perception that HIV is under control in the US, that it's Africa that has the

Despite the heavy perception that HIV is under control in the US, that it's Africa that has the problem, it's just not true. We are a hotspot.

–Shannon Hader

problem, it's just not true. We are a hotspot. We have a high rate of HIV across our city."

The district also boosted the number of HIV tests from 16,776 in 2004 to 92,748 in 2009—a dramatic 368% increase. In 2008, the district also instituted a syringe-exchange program that removed 130,000 used needles from the streets during its first six months and referred more than 40% of the 900 clients who used the program to drug treatment centers. Condom distribution also increased by more than 130% between October 2008 and July 2009, when roughly 2.3 million condoms were handed out at sites ranging from barbershops and nail salons to restaurants and bars.

The severity of the epidemic in Washington, D.C. has also prompted some unusual collaboration between the DC Department of Health and the National Institute of Allergy and Infectious Diseases (NIAID), a division of the US National Institutes of Health (NIH). In D.C., about a 30-minute drive from NIAID's headquarters in Bethesda, Maryland, NIAID has partnered with the GWU School of Public Health and the DC Department of Health to study what Carl Dieffenbach, the director of NIAD's Division of AIDS, describes as "the strongest, most complete example of a domestic urban epidemic in the US."

The collaboration has translated into a number of projects that could have wide-ranging impact on control and prevention of HIV, both locally and nationally. Notably, the NIH allocated $26.4 million to form the D.C. Partnership for HIV/AIDS Progress, a two-year research collaboration between the NIH and the DC Department of Health that is designed to find ways of reducing infections, improve the health of D.C. residents living with HIV/AIDS, and strengthen the response to the epidemic. The money is being funneled through both NIAID and the Office of AIDS Research, which oversees all government research dollars for HIV/AIDS.

NIAID, through its collaboration with the D.C. Partnership for HIV/AIDS Progress, is also conducting two multi-site observational studies with the HIV Prevention Trials Network (HPTN). In HPTN 061, a two-year study that began last year, trial investigators are collecting sexual and social networking information from black MSM. Volunteers in HPTN 061 are tested for HIV and other sexually transmitted infections, and asked about HIV risk behaviors, substance abuse, mental health problems, and homophobic violence to assess the impact these factors have on HIV incidence and related risk behaviors. HPTN 061 has enrolled men in six US cities, with 266 from the D.C. site. Manya Magnus, the DC site investigator for HPTN 061 and an associate professor at GWU, said this feasibility study's primary objective is to obtain information about the black MSM community that will be used to develop a much larger, community-wide, randomized, controlled trial to test the impact of behavioral and structural interventions.

A second observational study, HPTN 064, will attempt to estimate HIV incidence among high-risk women living in areas with high rates of poverty and HIV. HPTN 064 is also a two-year study and has enrolled women from six cities, including 210 from Washington, D.C.

Another proposed intervention referred to as test and treat, which calls for universal testing and immediate antiretroviral treatment for anyone who is HIV-infected, is also being studied in Washington, D.C. (see *Test and Treat on Trial*, *IAVI Report*, July-Aug. 2009). NIAID is now funding a study to test the feasibility of test and treat in one of Washington's high-risk communities (see *Vaccine Briefs*, *IAVI Report*, Sep.-Oct. 2009). The study, which began this summer, is also being conducted in the Bronx in New York City.

Rochelle Walensky, an associate professor of medicine at Harvard Medical School, who developed a mathematical model of test and treat in Washington, D.C., found that an expanded test and treat program would increase life expectancy of HIV-infected individuals. But her models showed only a modest impact on HIV transmission over the next five years (*Clin. Infec. Dis.* **51**, 392, 2010). Walensky thinks test and treat is a viable strategy in places like Washington, but worries that the public health community may have unrealistic expectations about what it can accomplish. "I wholeheartedly believe in frequent testing in high-risk populations and I wholeheartedly believe in universal treatment," says Walensky. "But I think we have to think of prevention as we think about antiretroviral therapy, chemotherapy, and the treatment of most other diseases. Test and treat isn't a magic bullet, but one page of the playbook."

Whether or not these interventions help the district gain control of the epidemic and bring about a dramatic decline in HIV incidence remains to be seen. "Despite the fact that it is grounded by trappings of wealth and ought to be a showpiece for the US, I cannot think of a more dramatic contrast or a starker example of the failure of public policy," says Fullilove. ■

Despite the fact that it is grounded by trappings of wealth and ought to be a showpiece for the US, I cannot think of a more dramatic contrast or a starker example of the failure of public policy.
–Robert Fullilove

MONKEY *MATTERS*

Nonhuman primate researchers gathered in New Orleans to discuss the growing number of models for HIV infection and pathogenesis

By Andreas von Bubnoff

Almost 200 participants gathered for this year's 28th Annual Symposium on Nonhuman Primate (NHP) Models for AIDS, held October 19-22 in New Orleans. The meeting was scheduled to take place here three years ago but was relocated to another primate center because of the devastating effects Hurricane Katrina had on the city.

Andrew Lackner, director of the Tulane National Primate Research Center (TNPRC), which hosted the conference, said New Orleans has rebounded quite strongly. "There are actually more restaurants in the city than there were before Katrina," he said. In addition to the opportunity to savor the fine restaurants of the Big Easy, attendees were also served a diverse selection of research updates, including insights from using NHPs as models for many different aspects of HIV infection and pathogenesis, as well as genetic characterization of NHP hosts.

Monkey models of transmission

An increasing number of NHP models are now available that can mimic different aspects of HIV transmission, and these models are important for testing vaccine, microbicide, or drug candidates.

An important component of these NHP models is the choice of challenge virus, and one characteristic of the challenge virus that is useful to understand is how sensitive it is to neutralization by antibodies.

To accurately predict the immunogenicity of candidate vaccines in challenge experiments, a challenge stock should ideally be moderately sensitive to neutralization by antibodies, said Katharine Bar, an assistant professor for infectious diseases at the University of Alabama at Birmingham. A challenge stock that is too resistant to neutralization—as appears to be the case for the commonly used challenge stocks simian immunodeficiency virus (SIV)mac239 and 251—might make it difficult to show protection even with good vaccine candidates, she said. Conversely, if a challenge stock is too sensitive to neutralization, a candidate vaccine that shows protection in animal experiments might not be protective when tested in humans.

Bar reported on the neutralization sensitivity of SIVsmE660, a challenge stock for which the neutralization sensitivity is still poorly characterized. "Characterizing the neutralization sensitivity of this virus will be helpful in figuring out if it's an appropriate challenge," said Bar. "Just understanding what we are dealing with is important for us to figure out how to interpret the results of past studies, and then going forward how to create a model that most closely recapitulates HIV-1." SIVsmE660 is similar to HIV in that it is a swarm, which means it contains many different virus variants. Bar and colleagues used single genome amplification (SGA) to isolate different variants from SIVsmE660 stocks, as well as the transmitted

founder viruses of animals rectally infected with SIVsmE660. They then determined the neutralization sensitivity of several variants to monoclonal antibodies directed to different parts of the SIV Env protein, and to serum taken from macaques that were infected with SIVsmE660. Preliminary results suggest that most variants are rather sensitive to neutralization, while others show intermediate or high resistance. This suggests that SIVsmE660 is a mix of variants with varying degrees of resistance to neutralization, and could explain some of the variable pathogenicity of this challenge stock after low-dose mucosal challenge.

Researchers are also trying to develop animal models of different viral transmission routes. Brandon Keele, head of the viral evolution and genomics core at the National Cancer Institute, gave an update on the development of an NHP model of penile HIV transmission he is working on in collaboration with Chris Miller at the University of California, Davis. In the model, the penises of male rhesus macaques were exposed to different doses of SIVmac251 by immersing their flaccid penis in a virus solution for up to 20 minutes; in some cases, the researchers also put a small amount of the solution onto the foreskin at the tip of the penis. According to Keele, SGA showed that all seven animals that were infected this way with three different doses (the lowest dose required multiple challenges) had just one transmitted founder virus, indicating this model of penile infection allows for single variant infections like most of the heterosexual transmissions of HIV in humans, Keele said.

At similar doses, vaginal infection of rhesus macaques is more likely to result in several transmitted founder viruses, the number of which is more likely to vary, Keele said. One possible reason for the difference could be that female rhesus macaques may be in different stages of their menstrual cycle. It is also possible that when immersing a penis in a virus solution, not all of the viruses in the solution touch the penis, whereas solution put inside the vagina might have more time to interact with the inner surface of the vagina. "You need a lot of virus to infect in the penile route," Keele said, "which means that the penile mucosa is a good anatomical barrier to infection and recapitulates human infection because you get a single infection, but it's difficult because you have to use a lot of virus."

Co-infection models

NHP models are also useful in modeling co-infection of HIV with other pathogens. A better understanding of HIV/tuberculosis (TB) coinfection in humans is especially important because TB is thought to be a leading cause of death among people with HIV/AIDS (see *Deadly Synergy, IAVI Report*, Sep.-Oct. 2009). In many cases, TB becomes latent when the TB bacteria hide in granulomas in places such as the lungs, but HIV infection, by weakening the immune system, can lead to reactivation of latent TB infection, said Smriti Mehra, a research scientist at the TNPRC, adding that she and her colleagues are developing the first HIV/TB coinfection model in rhesus macaques.

Mehra and her colleagues were able to induce SIV-induced reactivation of TB in rhesus macaques. They infected the macaques by letting them breathe in tuberculosis bacteria, which is how humans become infected. Once the infection had become latent for nine weeks, the researchers infected the animals intravenously with SIVmac239. They found that the animals developed clinical signs of TB within two weeks. The researchers want to use these animals to study the gene expression in the granulomas in the lungs and see which genes are expressed when TB is reactivated.

NHP models of HIV co-infection with sexually transmitted infections (STIs) are also important because STIs are associated with an increased risk and rate of HIV infection. Tara Henning, a postdoctoral fellow at the US Centers for Disease Control and Prevention (CDC), reported that she and her colleagues did the first successful triple infection of female pigtail macaques (PTMs) with the SIV/HIV hybrid SHIVSF162P3, the single-celled protozoan parasite *Trichomonas vaginalis*, and the bacterium *Chlamydia trachomatis*, with clinical presentation of genital STI symptoms that were similar to those observed in humans. An NHP model of STI-HIV coinfection would make it possible to investigate how STIs might contribute to enhanced susceptibility to HIV infection, and to test prevention strategies that target the genital mucosa in the context of STIs, Henning said.

Clues about pathogenesis

NHPs are also studied as a model of the pathogenic effects of HIV. One important hallmark of HIV infection is chronic immune activation. Structural damage to the gut is thought to contribute to this immune activation because of translocation of microbial products from the gut into the blood. Although the underlying mechanisms of this are still unclear, some clues are coming from studying the pathogenic effects of SIV infection in NHPs.

Photo by Andreas von Bubnoff

Nichole Klatt, a postdoctoral fellow in the lab of Jason Brenchley at the National Institute of Allergy and Infectious Diseases, reported on the identification of NK17 cells, a type of cell in the gut that can produce interleukin (IL)-17, a cytokine that is concentrated in mucosal tissues and produced in response to bacterial and fungal antigens. These cells had not been described before.

Klatt showed evidence that in SIV-infected rhesus macaques, loss of IL-17 producing $CD4^+$ T cells and $CD8^+$ T cells in the colon is associated with damage to the colon epithelium and with immune activation. However, loss of NK17 cells actually shows the strongest association with damage of the colon epithelium and with immune activation. This suggests a role for these IL-17 producing cells in maintaining the structural barrier of the gastro-intestinal tract and in preventing chronic immune activation, Klatt said.

Loss of NK17 cells in the gut might also play a role in causing the bacterial translocation and chronic immune activation that is often observed in PTMs, even if they are not SIV infected, according to Klatt (*Mucosal Immunol.* **3**, 387, 2010). Chronic immune activation is thought to be one reason why PTMs that are infected with SIV, such as SIVmac239, progress to disease more quickly than rhesus macaques. Klatt reported that in SIV-uninfected PTMs, only loss of NK17 cells, but not of IL-17 producing $CD4^+$ and $CD8^+$ T cells, was significantly correlated with damage of the colon epithelium. This suggests an important role for NK17 cells in the maintenance of the tight epithelial barrier of the colon, Klatt said. "Taken together, this study really is one of the first potential mechanisms showing why or how this damage may possibly occur during SIV pathogenesis," she added.

Controlling infection

Researchers are also studying NHPs infected with live-attenuated versions of SIV as a model for how the immune system can control viruses like HIV. One live-attenuated SIV that has been studied for many years is SIVmac239Δ*nef*, which has a deletion in the *nef* gene. Andrea Jordan, a research specialist in the laboratory of James Hoxie, a professor of medicine at the University of Pennsylvania, reported on studies with another live-attenuated version of SIVmac239 called ΔGY, where just two amino acids are deleted from the cytoplasmic tail of the Envelope protein.

Given that PTMs progress to disease more quickly than rhesus macaques when infected with SIVmac239, it was surprising that Jordan reported that PTMs infected with ΔGY control this live-attenuated virus better than rhesus macaques. The researchers found that all nine PTMs they had intravenously infected with ΔGY had undetectable or extremely low viral loads for several months to years, whereas in ΔGY-infected rhesus macaques, viral loads were low, but still detectable in all cases. In addition, ΔGY-infected PTMs were not only protected from homologous intravenous challenge with SIVmac239, but could also bring viremia from a heterologous intravenous challenge with SIVsmE660 to very low or undetectable levels. In contrast, SIVmac239Δ*nef* only provides partial or poor control from intravenous heterologous E660 challenge in rhesus macaques, Hoxie said.

This suggests that ΔGY-infected PTMs could serve as an interesting model system to identify immunological correlates of protection. "We have a very interesting puzzle here why an animal species that is prone to early deaths and AIDS faster than the rhesus appears to be our preferred model for complete control and protection with ΔGY," Hoxie said. "We've made a very small change and have totally changed pathogenesis. We've rendered a highly pathogenic virus to be totally controllable."

When the researchers depleted the $CD8^+$ T cells in the ΔGY infected PTMs, they found that this led to reappearance of ΔGY, after two years in which ΔGY RNA was undetectable in plasma, suggesting that cellular immunity has a role in the protection, although it's also possible that antibody-dependent cellular cytotoxicity (ADCC) plays a role, Jordan and Hoxie said. Hoxie said the ΔGY-infected PTMs also resemble elite controllers, HIV-infected individuals who control virus replication below detectable levels without treatment, because ΔGY initially replicates similar to wild type levels of SIVmac239 but is later rendered controllable by host immune responses.

Cristian Apetrei, an associate professor of microbiology and molecular genetics at the University of Pittsburgh, described the development of another NHP model of elite controllers. Apetrei and colleagues intravenously infected five

Indian rhesus macaques with SIVagm, the SIV that naturally infects African green monkeys without causing disease. The SIVagm-infected rhesus macaques resembled human elite controllers, in that they had undetectable viral loads for four years, completely recovered levels of mucosal $CD4^+$ T cells by four years after infection, and no disease progression. Depletion of $CD8^+$ T cells four years after infection resulted in a transient rebound of viral loads, suggesting that SIVagm was controlled by the immune system. Apetrei said that having such a model is important because it makes it possible to study how elite control develops early after infection.

SNPs and genomes

Another theme of this year's meeting was efforts to genetically characterize NHP hosts. When it comes to the availability of genetic tools, NHPs have a long way to go compared to mice or fruit flies, according to Jessica Satkoski Trask, a postdoctoral research associate at the University of California, Davis. "If you want a knockout mouse, you call the knockout mouse store and they send you the mice," she said, referring to mice that have a gene knocked out. In contrast, "rhesus macaques and primates are not set up as a genetic model yet. There is going to be a need for a more and more diverse set of genetic tools."

One such tool is called single nucleotide polymorphisms (SNPs), variations in the genetic code that geneticists use to find candidate genes for certain genetic traits or phenotypes. This could be important to identify, for example, the genetic factors that are the basis for different responses of Chinese and Indian rhesus macaques to infection with SIV.

Traditionally, most studies that use NHPs as a model for HIV infection have used Indian rhesus macaques, and Chinese rhesus macaques are also gaining in popularity, Satkoski said. However, she said, until recently just a few hundred SNPs were known for Chinese and Indian rhesus macaques, while over 25 million are known for humans and about 14 million for mice. Now Satkoski and colleagues have identified more than 4,000 additional SNPs in Indian and Chinese rhesus macaques that are unique and consistently observed with different frequencies in the two groups. They also made a linkage map, which means that they determined where those SNPs are located on the chromosomes and which SNPs are likely inherited together.

The availability of more SNPs on a linkage map will accelerate the process of finding candidate genes for inherited traits, Satkoski said. She and her colleagues have already used their more detailed SNP linkage map to identify some candidate genes, including one that likely has an impact on elite controller status in Chinese rhesus macaques.

The newly identified SNPs will also allow researchers to screen animals that are going into research protocols, allowing them to determine the degree to which an animal is really a Chinese and not an Indian rhesus macaque. In the future, it may also be possible to use the new SNPs to determine the likelihood that a Chinese rhesus macaque is going to be an elite controller. "[This] will allow you to have a more uniform research population," Satkoski said.

Chinese rhesus macaques might be the more relevant model for AIDS in humans than Indian rhesus macaques because they don't progress to AIDS as quickly, said Bianca Mothé, an associate professor of biology at the California State University San Marcos. In addition, a larger percentage of Chinese rhesus macaques become elite controllers, Mothé said. One possible explanation for the different disease progression in Chinese rhesus macaques could be their MHC class I alleles. MHC class I alleles are important for the cellular immune response because antigen presenting cells use MHC class I to present antigens to $CD8^+$ T cells, but so far these MHC alleles have not been well characterized in Chinese rhesus macaques.

Mothé and colleagues sequenced the MHC class I alleles of 50 Chinese rhesus macaques from different primate centers and found that Chinese rhesus macaques have a more varied MHC composition than Indian rhesus macaques. In addition, when Mothé and colleagues predicted the peptides that can be bound by these MHC alleles, they found that three of the four most frequent MHC alleles in Chinese rhesus macaques bind the same peptides as the three most common HLA motifs in humans. Together, this suggests that Chinese rhesus macaques are more similar to humans in their MHC class I alleles than Indian rhesus macaques, which may explain why Chinese rhesus macaques are more similar to humans than Indian rhesus macaques in their disease progression to AIDS (*Immunogenetics* **62**, 451, 2010). ■

Vaccine BRIEFS

Efficacy Trial Shows Combination of Two Antiretrovirals Can Prevent HIV Infection

A STUDY OF NEARLY 2,500 MEN and transgendered women who have sex with men at 11 clinical sites in the US, South Africa, Brazil, Thailand, Peru, and Ecuador showed that daily administration of the antiretrovirals (ARVs) emtricitabine (FTC) and tenofovir (TDF) was 44% effective in protecting men or transgendered women who have sex with men from HIV infection, according to a study published in November (*N. Engl. J. Med* . doi: 10.1056/NEJM0a1011205). This randomized, double-blinded, placebo-controlled study, known as iPrEx, is the first efficacy trial of oral pre-exposure prophylaxis (PrEP) to be completed.

Many researchers heralded the success of PrEP in this trial as an important development in HIV prevention research, a field that after years of gridlock has seen much progress. In September 2009 researchers reported results from the RV144 trial in Thailand that showed a prime-boost vaccine regimen provided about 31% protection against HIV infection. Then in July, microbicide researchers reported that vaginal application of a 1% gel formulation of tenofovir was 39% effective in blocking HIV infection.

The iPrEx results were statistically significant with a confidence interval that ranged from 15 to 63. In the group receiving ARVs, either TDF or FTC was detected in 22 of 43 seronegative volunteers (51%), and in only three of 34 volunteers (9%) who acquired HIV during the course of the study, indicating that detectable quantities of either of the study drugs in the blood was strongly correlated with the protective effect.

The news of the efficacy of PrEP did not come as a surprise to many researchers in the field. For years, researchers have suspected that the life-extending ARVs that have proven so successful in treating HIV/AIDS might also be useful in prevention. The first study in non-human primates that hinted this might be a promising approach for HIV prevention was done nearly 15 years ago (see *PrEP Work*, *IAVI Report*, Nov.-Dec. 2008). A study by Gilead, the pharmaceutical company that developed both TDF and FTC, showed that TDF was effective at preventing infection with simian immunodeficiency virus (SIV), the monkey equivalent of HIV, in rhesus macaques. Since then, several monkey experiments have shown that FTC-TDF was even better at protecting against SIV infection in models that more closely recapitulate HIV transmission.

One of the obstacles to implementing PrEP as an HIV prevention strategy will be adherence—for the drug to work, individuals at risk of HIV infection must take it faithfully. Among individuals in the FTC-TDF group who had detectable drug levels in their blood, the odds of HIV infection were 12.9 times lower, corresponding to a 92% reduction in risk of HIV infection as compared to volunteers in the FTC-TDF group who did not have detectable levels of the drugs in their blood.

In the iPrEx trial, all volunteers were counseled on a monthly basis to adhere to the daily dosing regimen. Investigators collected self-reported information on adherence as well as pill counts at these monthly visits. However, blood levels of the drugs, measured by an intracellular assay that was expected to detect TDF 14 days or more after the last dose was taken, indicated self-reported adherence was not an accurate measure of how often volunteers had actually taken the pill. “Although reported pill use was high, drug exposure that was measured objectively was substantially lower,” the study’s investigators noted.

The investigators speculate that side effects, including nausea and unintended weight loss, associated with initiation of FTC-TDF may have contributed to the overall low adherence to the study drugs. There was also a trend toward more elevated serum creatinine levels, which are known to impair renal function, in the FTC-TDF group than in placebo recipients. Although this side effect appeared to reverse upon discontinuation of FTC-TDF and only occurred in a small subset of volunteers, Nelson Michael, director of the US Military HIV Research Program, who wrote an editorial in the *New England Journal of Medicine* on the iPrEx study, concluded that “this finding raises both safety and monitoring concerns regarding possible cumulative toxic effects associated with large-scale exposure to daily FTC-TDF therapy for an extended period.” He also cautioned that the side-effect profile for FTC-TDF was probably diluted in this study because of low adherence to the drugs, suggesting the side effects could be even more substantial in volunteers who were compliant to the daily regimen.

In addition to side effects, another concern with PrEP is the potential for development of drug resistance if a person unknowingly becomes HIV infected and continues taking ARVs for

PrEP. In the iPrEx study, none of the volunteers in the FTC-TDF group or the placebo group who became HIV infected during the course of the trial developed drug resistance. Two volunteers who were infected at enrollment but placed on FTC-TDF anyway, did develop FTC-resistant virus. Researchers speculate that the lack of any drug resistance may have been due in part to the overall low adherence to the study drugs.

Now that PrEP has also shown partial efficacy against HIV, there are certainly many thorny issues that will need to be resolved before it can be implemented, particularly in developing countries that continue to bear the greatest burden of HIV infection. With only a third of HIV-infected individuals in need of HIV therapy currently receiving it, there is bound to be some debate about how to dole out antiretrovirals for prevention. Robert Grant, an associate professor of medicine at the Gladstone Institute of Virology and Immunology and the principal investigator of the iPrEx study, says researchers must still address whether FTC-TDF is as effective in preventing HIV infection in other high-risk populations, such as injection drug users or women in areas of high HIV prevalence.

Grant says it will also be important to determine if daily dosing is needed, or whether intermittent dosing before and after sex will be sufficient to protect against HIV infection. "I think the durability of how long people can use this is also an open question," says Grant. "The median duration of follow-up in the iPrEx trial was 1.2 years, the maximum was 2.8 years. It is conceivable though that people might want to use [FTC-TDF] for a longer period of time."

Grant says there are also questions about whether using ARVs for prevention is economically feasible, even in rich countries. "There is no mechanism for reimbursing individuals for the cost of [using ARVs] as a preventive measure, which could dissuade potential users," he adds.

Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases (NIAID), a funder of the study, says the iPrEx results are encouraging, but "in no way lessen the need for a vaccine, which would be a gigantic step in the fight against AIDS," says Fauci.

In the meantime, the iPrEx trial results have prompted discussions among AIDS vaccine investigators and advocates over whether volunteers should be counseled and given access to PrEP in addition to the other risk-reduction methods that are currently standard in AIDS vaccine trials. There are even discussions about whether ongoing vaccine trials, such as the HVTN 505 trial of 1,350 men who have sex with men (MSM) in the US, should be adapted to include a PrEP arm.

Scott Hammer, principal investigator of the HVTN 505 trial, says US regulatory authorities should provide some clarity soon about if or how PrEP should be used. The US Centers for Disease Control and Prevention is expected to publish interim guidelines for healthcare providers in the coming weeks, followed by formal US Public Health Service guidelines. In the meantime, Hammer says an effort is underway by trial sites to address questions that the MSM community has regarding the iPrEx results.

The HVTN 505 trial is testing the safety and efficacy of a DNA/adenovirus serotype 5 prime-boost regimen developed at NIAID's Vaccine Research Center. Volunteer enrollment in the HVTN 505 trial was slow at first, but the results of the iPrEx trial may actually have boosted the MSM community's interest in vaccine trials, Hammer says. —*Regina McEnery and Kristen Jill Kresge*

Latest UNAIDS Report Shows Significant Advances in Combating HIV/AIDS

In its annual report on the status of the pandemic, the Joint United Nations Programme on HIV/AIDS (UNAIDS) gave everyone something to cheer about when it declared that the world has "halted and begun to reverse the spread of HIV."

The 364-page report, released in advance of World AIDS Day on Dec. 1, highlighted several bright spots. Over the last decade, HIV incidence declined by more than 25% in 33 countries, 22 of them in sub-Saharan Africa. Globally, HIV incidence declined by 19% between 1999, when incidence likely peaked, and 2009.

Another area of progress was the continued scale-up of antiretroviral drugs in low- and middle-income countries. An additional 1.2 million people in low- and middle-income countries received antiretroviral therapy in 2009, bringing the total to 5.2 million, a 30% increase over 2008. Despite this increase, only 36% of the people who are eligible to receive antiretroviral therapy in developing countries at the end of 2009 were receiving it. Significant progress has also been made in preventing mother-to-child transmission of HIV and in reducing HIV-related stigma and discrimination.

The report also highlighted some major obstacles in combating HIV/AIDS, notably the alarming trends in Eastern Europe and Central Asia, where HIV incidence has increased by more than 25% between 2001 and 2009, a jump that UNAIDS attributes to both a lack of access to services and anti-drug use laws. Also, the global economic recession is seen as a major threat to HIV/AIDS treatment and prevention programs in developing countries.

In a foreword to the report, UNAIDS executive director Michel Sidibé said the world should be proud of the recent accomplishments in the fight to end AIDS, but cautioned that it is too soon to say mission accomplished. "Growth in investment for the AIDS response has flattened for the first time in 2009. Demand is outstripping supply. Stigma, discrimination, and bad laws continue to place roadblocks for people living with HIV," said Sidibé. —*Regina McEnery*

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