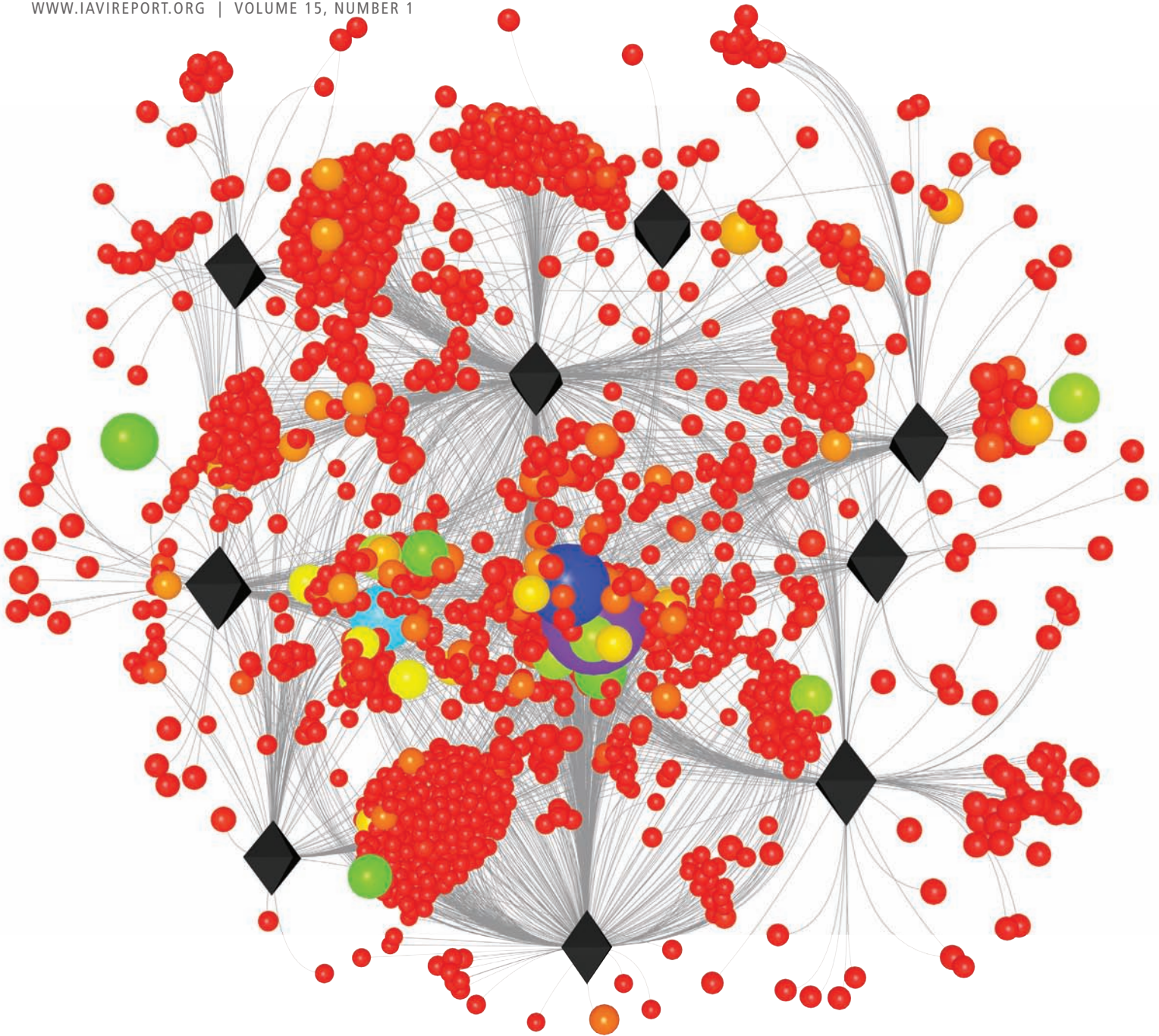


January-February 2011

IAVI Report

The Publication on AIDS Vaccine Research

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**EXPERTS ON
ANTIBODY ADVANCES**

**PLUS
Labors of Love:
HIV Research Couples**

EDITOR'S LETTER

THIS YEAR MARKS 30 YEARS since the first published description of AIDS in the US, an anniversary that will likely spur considerable reflection on the advances in both treating and preventing HIV/AIDS.

A notable area of recent progress in vaccine research is the isolation of several HIV-specific broadly neutralizing antibodies. In this issue, we feature the second installment of our Living History of AIDS Vaccine Research series (see *As Antibody Findings Mount, What Comes Next?*, page 4). This time we discuss the recent antibody advances with four leading experts to add some perspective to these findings and to the challenges in turning these discoveries into HIV vaccine candidates.

In a related story, we summarize the key findings of a recent study that describes the development of broadly neutralizing antibodies in HIV-infected individuals in greater detail than previous studies (see *Research Briefs*, page 23).

Despite a three-decade-long effort to understand the biology of HIV and how the virus is transmitted, there are still unanswered questions that some researchers think could be impeding the development of effective vaccines and microbicides. One question is whether HIV-infected cells, in addition to free virus particles, contribute to HIV transmission. In *Is HIV Hitching a Ride Inside Cells?* (see page 8), we review what is known about cell-bound HIV transmission and why it might matter.

We never shy away from tackling complex topics at *IAVI Report*, whether it's somatic hypermutation or the latest developments in structural biology. But in this issue we take on a subject we've never broached before, matters of the heart. That's right, love.

We've been interested for some time in the numerous couples that are both involved in HIV research, oftentimes working side by side in the lab or as co-investigators on trials. And when we began thinking about writing a story on this, we collected a long list of couples working on HIV research. In this issue, we present the stories of how science and romance intermingle for four of these couples (see *Chemistry Lab*, page 12).

That's not the only first in this issue. We are happy to also feature our first Commentary piece, authored by veteran vaccine developer Stanley Plotkin, who wishes to remind HIV vaccine researchers that vaccine development has not always been done empirically (see *More than Trial and Error*, page 17). Another new foray in this issue is a review of two recently published books that detail the roots and repercussions of the anti-vaccine movement (see page 19).

We hope you will enjoy reading these new features and, as always, we welcome your feedback and ideas.



KRISTEN JILL KRESGE



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The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996 and operational in 25 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI was founded with the generous support of the Alfred P. Sloan Foundation, The Rockefeller Foundation, The Starr Foundation, and Until There's A Cure Foundation. Other major supporters include 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 Governments of Canada, Denmark, India, Ireland, Japan, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government (Spain), the European Union as well as the National Institute of Allergy and Infectious Diseases and The City of New York, Economic Development Corporation; multilateral organizations such as The World Bank and The OPEC Fund for International Development; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, The Gilead Foundation, GlaxoSmithKline, Google Inc., Pfizer Inc, and Thermo Fisher Scientific Inc.; leading AIDS charities such as Broadway Cares/Equity Fights AIDS; and many generous individuals from around the world. For more information, see www.iavi.org.

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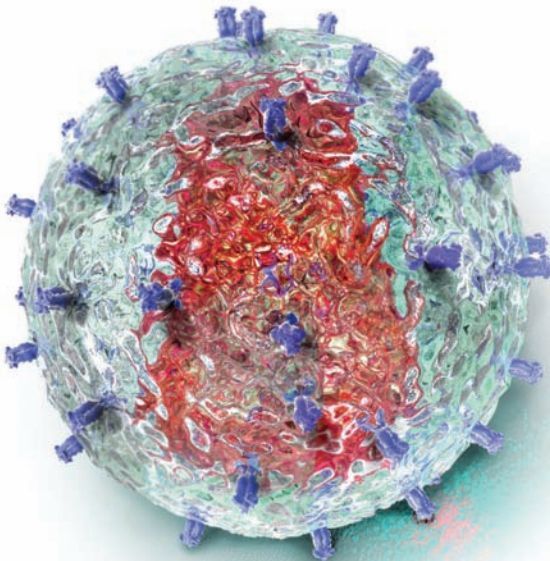
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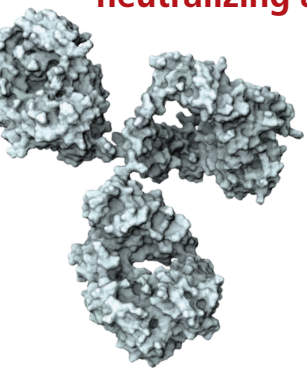
[ON THE COVER]

Visualization of the HIV-host protein interaction network. Colored spheres and black diamonds correspond to human and HIV-1 proteins respectively, with grey lines corresponding to one or more unique protein interactions (Dickerson et al. 2010, *BMC Syst. Biol.* 4, 80, 2010). Sphere diameter and color indicate the human protein connectivity—larger and cooler color indicates more interactions.

Jonathan Dickerson and David Robertson,
University of Manchester, UK

As antibody findings mount, what comes next?

Four leading researchers assess the field and what it will take to get from broadly neutralizing antibodies to immunogens



In 2009, the first report of new and more potent HIV-specific broadly neutralizing antibodies kicked off something of an antibody frenzy.

Since then, researchers have isolated nearly two dozen broadly neutralizing antibodies from HIV-infected donors. These antibodies

are vital clues for HIV vaccine development and are now being used by researchers to try to reverse engineer vaccine immunogens. In this second installment of IAVI Report's Living History of AIDS Vaccine Research, Managing Editor Kristen Jill Kresge and Science Writer Regina McEnery turned to four experts to provide some perspective on these recent advances in antibody research and to frame the current efforts to design vaccine candidates that can induce antibodies against HIV.

Do you feel more optimistic now about AIDS vaccine development? If so, why?

Dennis Burton: I feel much more optimistic about the possibilities for an AIDS vaccine than I have for probably a decade. This is primarily because we're seeing a lot more of the sorts of antibodies that we'd like to induce through a vaccine than we've ever seen before. For 10 to 15 years we've been working with a handful of antibodies and we really thought maybe they were a very special beast and we would not be able to induce them via a vaccine. But in the last year or so we've identified, between the different groups, something like 20 new such antibodies, and they're a vital clue to how to make an AIDS vaccine. I think this is a tipping point in a way. The next two, three, four years will tell us a lot about the feasibility of an HIV vaccine.

John Mascola: I'm absolutely more optimistic. I think the isolation of numerous monoclonal antibodies, the fact that the immune system does this pretty routinely, and the fact that we have much better tools to measure what we're doing, all suggest to me that the problem is solvable in due time. In science, you just can't predict how long that is, but I would say I'm quite optimistic now.

What is special about the new antibodies that have been isolated recently from HIV-infected donors?

Burton: The most significant factor is that these new antibodies are probably 100 times better than the previous broadly neutralizing antibodies. Some of them hit 90% of the world's [HIV] isolates. They're also very potent, and the more potent they are, then the less of the antibody we'll have to induce through vaccination, so we like potency a great deal.

Mascola: What makes VRC01 [an antibody discovered by researchers at the Vaccine Research Center] special is what we call a precise mode of targeting. It finds the spot on the virus that is unable to really mutate or change because it's the initial part of the virus that binds CD4. Therefore, this site has to be exposed and the antibody is able to access it. The fact that we can define this very precise region of the virus that is attacked by the antibody means we can express that very precise region in a number of ways, and so it gives us the opportunity to try a number of different approaches to vaccine design. While we understand the structure very well, when it comes to knowing what the immune system's going to do, the science really is in the experimental testing.

So how do you get from these antibodies to vaccine immunogens?

Burton: A number of different strategies are being pursued to try and work backwards from the antibodies to immunogens, a

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Nelson Michael

process we call reverse engineering. We are taking pieces of the virus that we see are recognized by the antibodies and using those. We are altering the surface of the virus proteins, or the surface of the virus even, based on what we know about how the antibodies bind to the virus.

The target of all neutralizing antibodies against HIV is a complex of proteins on the surface of the virus known as the trimer. This is quite an instable structure, which is one of the problems in trying to make a vaccine. One way around this is to forget about the trimer altogether and to use pieces of the trimer that are recognized by the broadly neutralizing antibodies. An alternative is to really try and make the trimer. If you do it right, if you can really stabilize the trimer, then all the broadly neutralizing antibodies should bind the trimer and in theory [the trimer should] elicit all of the relevant antibodies. Stabilizing the trimer is a great problem. Perhaps one of the best advances in trying to stabilize the trimer would be if we had its structure, but we're in a sort of vicious circle here. We can't get its structure because we can't get it stable enough, and we can't get it stable enough because we can't get the structure. So now people are trying to bootstrap their way towards the structure.

How challenging is it to reverse engineer immunogens based on these new broadly neutralizing antibodies?

Mascola: There certainly have been examples of vaccines that have been thoughtfully and rationally designed, but we are in uncharted territory in the level of sophistication, I think, that we have to attain to really rationally approach the types of immunogens we need, and the type of vaccine strategies we need. I think it's a tougher problem than has been approached before.

Burton: Reverse engineering of this type really started largely out of work with HIV. One of the difficulties with the whole reverse engineering strategy is it seems these antibodies are often quite picky about how they recognize the virus, so it means that the immunogens may have to be designed quite precisely and that's very challenging.

The hope with the new antibodies is that they are more effective so that might give us a little bit more leeway in how well we design the immunogens. But we don't have the sites defined well enough at this moment to say whether immunogen design is going to be easier, the same, or harder for the new antibodies. I think that's one of the issues that's going to be resolved in the next two years or so. We do know that quite a few different infected individuals have made antibodies to these sites, so they're not completely intractable. That's cause for hope but there are many, many unknowns still that we need to work out.

If reverse engineering could be made to work, I think it would be very exciting. It would be tremendous for vaccines generally

THE PANELISTS



DENNIS BURTON is a professor of immunology and microbial science at The Scripps Research

Institute (TSRI) in La Jolla. Burton oversees IAVI's Neutralizing Antibody Center at TSRI, which figured prominently in the discovery of several of the new broadly neutralizing antibodies, including two called PG9 and PG16.



JOHN MASCOLA is the deputy director of the Vaccine Research Center at the US National Institute of

Allergy and Infectious Diseases, a division of the US National Institutes of Health. He was part of a team of researchers that isolated a few of the new broadly neutralizing antibodies, including VRC01.

because it would reduce the problem to simply finding some good antibodies and then working backwards.

What is known about whether any of the broadly neutralizing antibodies can actually protect against HIV?

Burton: We believe that they would protect against HIV because they do so in the monkey model. These antibodies, once individuals are infected, don't seem to affect the course of disease very much. They work much better if they're present before the virus.

Julie Overbaugh: There have certainly been a lot of examples in nonhuman primates that you can protect against HIV using HIV-specific monoclonal antibodies in very, very experimental settings. But in humans, the data is pretty sparse. Probably the best data comes from mother-infant transmission studies. There's only been one study done in infants that I'm aware of, but that's the closest thing to a vaccine setting. And in that study that we recently completed, which is not yet published, we didn't see any evidence, no matter how we looked, of a benefit of having those antibodies in the infants. It doesn't preclude the idea that neutralizing antibodies could work, if they're the right ones, if they're broad enough, if they're targeted to the right viruses, and if there's enough of them. But I think it says that this bar is actually quite high in terms of eliciting neutralizing antibody responses.

Mascola: What it will take for an antibody to protect is something we need to keep a very open mind about. I think for an optimally effective vaccine—for a vaccine that really does prevent most infections, most of the time, against most viruses—it's likely to require some level of virus neutralization. But we don't really know what level of neutralization is going to be required in humans.

So are passive immunization studies with the new antibodies something the field should consider?

Mascola: I think the idea of getting proof of concept in humans



NELSON MICHAEL is the director of the US Military HIV Research Program, a key collaborator in the

RV144 efficacy trial in Thailand of a prime-boost vaccine regimen (a canarypox vector based candidate, ALVAC-HIV, followed by a recombinant protein, AIDSVAX gp120 B/E) that demonstrated about 31% efficacy in preventing HIV transmission.



JULIE OVERBAUGH is the associate program head at the Fred Hutchinson Cancer Research

Center in Seattle. She studies the biology of HIV and transmission of the virus, particularly vertical transmission from a mother to her infant.

that antibodies can protect is critical. It would provide sort of a framework for the field to say we know these types of antibodies protect and therefore we really have to work at eliciting them. We have had a lot of discussions at the Vaccine Research Center with a whole group of colleagues in the field about the potential for making clinical grade antibody and for doing clinical trials. Right now, it is our intention to pursue that course and to work with two groups of clinical cohorts, potentially. One would be high-risk adults and the other is the setting of maternal-to-child transmission of HIV.

We would probably expect this approach to work but if it didn't, it would maybe make us rethink some of the conventional wisdom about amounts of antibody and mucosal immune responses, for example.

Overbaugh: I think testing those antibodies in adults who are highly exposed or in pregnant mothers is an interesting way to examine the question. I would say that doing that in the setting of mother-to-child transmission is now very complicated because we know that taking antiretroviral drugs, either mother or infant, is highly protective. So that will be the benchmark against which these antibodies would have to be tested, and that might make for a very large trial to be able to see any efficacy. But in high-risk populations it may be interesting to look at those antibodies perhaps in mixtures. I don't think we really know how much antibody is even likely to be protective.

Is it possible that antibodies explain the partial protection seen with the prime-boost vaccine regimen tested in the RV144 trial in Thailand?

Nelson Michael: We certainly have confirmed in RV144 that we don't generate very much in the way of broadly, cross-reactive neutralizing antibodies, which is generally seen in our field as the Holy Grail. What we did see [in RV144] is a very strong amount

of binding antibody and a very high level of what we call ADCC [antibody-dependent cellular cytotoxicity]. ADCC is an important effector mechanism of antibodies. If I had my druthers, I would have much rather seen that plus neutralizing antibodies because I would wager that if we had a vaccine that could do that, we'd all jump on it.

So do the RV144 results indicate that non-neutralizing antibodies can play an important role in protecting against HIV?

Burton: There is some emerging evidence that under certain circumstances, at high concentrations, non-neutralizing antibodies can impart some partial protection. This may be contributing to the results of the Thai trial, which do seem to indicate some very, very modest protection. So there is interest that maybe mechanisms other than neutralization can contribute to protection. As to whether they really will, under normal circumstances, is a topic for investigation.

I think it's worth investigating the protective activities of non-neutralizing antibodies. It is not a new phenomenon to find non-neutralizing antibodies can act against viruses and afford some protection. Generally, the protection afforded by such antibodies is much less effective than neutralizing antibodies.

Do you think it would be possible to develop a highly effective HIV vaccine without inducing broadly neutralizing antibodies?

Burton: I think many of us feel that without broadly neutralizing antibodies, a vaccine would be difficult, if not outright impossible. It's for good reason that virologists focus on neutralizing antibodies. But of course if all the problems prove intractable then there's a case to be made for saying, well, okay, we have failed on the neutralizing, but let's try and induce a high level of non-neutralizing antibodies.

Overbaugh: I still think we're going to need multiple components to this. Neutralizing antibodies that are very, very broad and are targeted to the best possible epitopes may be part of it. My gut feeling is that will not be adequate and there will have to be something else that will have to contribute to vaccine efficacy.

Michael: I think it's going to be difficult for us to understand what it really is going to take, frankly, to make a vaccine that generates broadly, cross-reacting neutralizing antibodies. Some of the best minds in the field are on that. I would say that it's exciting that maybe we can develop a licensed vaccine that will hold the line for that blessed moment when we're able to reverse engineer vaccines that will actually make broadly neutralizing antibodies. My view is that, once we're able to do that, you're going to be looking at efficacies that are very, very high and it will be that wonderful part of my career when I can think about working on malaria. ■

Is HIV Hitching a Ride INSIDE CELLS?

Despite great progress in understanding HIV transmission, researchers still don't know if it is primarily the result of free virus particles or HIV-infected cells

By Andreas von Bubnoff

MORE THAN 25 YEARS after the discovery of HIV, researchers have made great strides in understanding many aspects of HIV transmission. However, one fundamental question remains unanswered: What role, if any, do HIV-infected cells play in HIV transmission?

“This is an understudied topic in the field,” Deborah Anderson, a professor of obstetrics/gynecology and microbiology at Boston University School of Medicine, recently wrote in a review article (*AIDS* 24, 163, 2010). Anderson became a champion for the importance of understanding cell-associated HIV transmission over 25 years ago, when she first coined the term “Trojan Horse leukocytes,” to illustrate the possibility that the infectious agent later identified as HIV could enter a person's body hidden inside a white blood cell, in which case it would be shielded from many of the body's antiviral defense mechanisms (*N. Engl. J. Med.* 309, 984, 1983).

Anderson has done many studies to characterize HIV-infected cells in semen and cervicovaginal secretions, showing, she says, that they are often present in high numbers and are highly infectious. She says that even though HIV-infected cells in genital secretions—especially infected macrophages and CD4⁺ T cells—may play an important role in the sexual transmission of HIV, they have been largely overlooked in recent studies of the mechanisms of HIV transmission, and in the design and testing of HIV vaccine and microbicide

candidates. In addition, she says, most nonhuman primate (NHP) challenge models use cell-free virus stocks to test vaccine and microbicide candidates, which is why the candidates may not protect against cell-associated viral transmission. This, she adds, may explain the failure of several vaccine and microbicide candidates in recent clinical trials. “It's really interesting that there is this blind spot,” she says. “People are so invested with their models now that are based on the cell-free system that they are, I think, reticent to branch out.”

Others agree that knowing the role of HIV-infected cells in HIV transmission is important. “It is a basic question, and it is rather amazing that we haven't answered it yet,” says Grace Aldrovandi, a professor of pediatrics at the Children's Hospital in Los Angeles. “We do kind of need to know what we are targeting,” says Julie Overbaugh, a member at the Fred Hutchinson Cancer Research Center. “If it's really cell-associated virus that's important, then maybe we want immune factors that can target and lyse the infected cell, whereas if it's cell-free virus, then maybe we want things that are more effective in cell-free virus.” One concern is that cell-associated transmission, if it plays a role, might be harder to prevent. “[For] cell-associated virus [it's going to be] harder to be able to intervene with things like antibodies and things that would require being able to actually have access to the virus,” says Jairam Lingappa, an associate professor of global health and medicine and an adjunct associate pro-

fessor of pediatrics at the University of Washington.

One major reason the role of cell-associated transmission is still unclear is that it is difficult to study. Researchers have analyzed genital fluids and breast milk for the presence of HIV-infected cells, and have studied the role of these cells in sexual or mother-to-child transmission (MTCT) of HIV. But so far, no clear consensus has emerged from these studies about the role of cell-associated HIV in transmission.

Understanding sexual transmission

Most HIV infections are the result of sexual transmission of the virus. Semen can contain free HIV particles, as well as white blood cells such as CD4⁺ T cells, which can be HIV infected and therefore may contribute to transmission. But so far, only a few studies have addressed the relative contribution of HIV in the cell-free and cellular portions of the semen to HIV transmission. Such studies typically separate the semen into the cellular and the cell-free fractions. They then analyze the HIV DNA sequences of the HIV-infected cells (such as HIV provirus integrated into the genome of the infected cell) in the cellular fraction, and the HIV RNA sequences of the free HIV particles in the cell-free fraction.

In 1996, David Ho and colleagues were the first to look at this issue in five men who have sex with men (MSM) transmission pairs (*J. Virol.* 70, 3098, 1996). They compared the HIV sequences of the cellular and cell-free fractions of the donor's semen with the sequences in the blood plasma of the recipient. They found that in three cases, the cellular fraction of the donor's semen was more similar, and in one case the cell-free part was. This was consistent with both cell-associated and cell-free HIV playing a role in transmission, Ho says. However, he adds, the study was of limited value because the samples were taken one to two months after transmission, which means that the HIV sequences in the donor could have changed in the meantime. "One cannot draw definitive conclusions," says Ho.

Last year, Davey Smith, an associate professor at the University of California in San Diego, and colleagues did sequence analysis of six MSM rectal transmission pairs a few months after infection. They reported that the HIV sequences in the cell-free part of the donor's semen were consistently more similar to the HIV in the recipient's blood than the sequences in the cell-associated part of the donor's semen. This suggested that cell-free and not cell-associated HIV is involved in

sexual transmission through semen (*Sci. Transl. Med.* 2, 18re1, 2010).

But then James Mullins, a professor of microbiology and medicine at the University of Washington, and colleagues re-analyzed the data and concluded that Smith and colleagues had included contaminated samples in their study because the HIV sequences in the cellular part of the donor's semen were too different to have come from the same donor as the HIV sequences in the cell-free part of the semen (*Sci. Transl. Med.* 2, 50le1, 2010). "What we found, and it's really unimpeachable, is that the cells were actually from different people," Mullins says. "That's why they weren't closely related [to the recipient's HIV]." Mullins says Smith and colleagues might have mixed things up in the laboratory, or samples coming into their laboratory were mislabeled. "They didn't figure it out by a long shot," adds Mullins. "In fact they have only made the field more confused."

Smith says he went back and did not find evidence of contamination, mixed up samples, or mislabeling, but acknowledges that he can't completely rule them out (*Sci. Transl. Med.* 2, 50lr1, 2010). He says his finding should be validated, which is why he is now trying to better characterize the cellular HIV sequences in the semen of additional transmission pairs. In addition, he says, not enough is known about sequences of HIV found in infected cells in semen to judge what degree of sequence diversity one would expect. "It's not that easy to find cellular HIV sequences, and very few people have actually done it," Smith says. "We really don't know how diverse that population actually is and we can't really just dismiss everything there just because we haven't seen it before."

But for now, the concerns about the study remain. "The problem is there are going to be a lot of people who are just not going to look at this as being very useful information because of the contamination issue," Lingappa says.

Mullins wonders if it will ever be possible to determine to what extent cell-associated HIV contributes to sexual transmission through semen. "I am not very optimistic," he says. One challenge, Mullins and others say, is to find transmission pairs where the partners are willing to give blood or genital fluid samples at a time close enough to transmission to be able to get meaningful results when comparing sequences. "It's hard to get the fluid, and especially hard to get the fluid at the right time," Mullins says. Even samples collected just a week after transmission might already have changed from the semen that caused the transmission, he adds.

One hint that HIV-infected cells might play a role in heterosexual HIV transmission is that as many as 20% of heterosexual transmissions involve more than one transmitted founder virus, says Ron Swanstrom, director of the University of North Carolina Center for AIDS Research. That's a much higher percentage than what would be expected if the infections were due to cell-free virus because the probability of infection with cell-free virus is thought to be 1% or lower, and so the combined probability of independent infections with more than one free virus would be expected to be even lower than that. An alternative explanation is that the infection occurs through cells that carry several proviral DNA copies of HIV, especially if additional sexually transmitted infections in the donor result in more HIV-infected cells that could be transmitted. However, it is also possible that infections increase the number of HIV target cells in the recipient, or that circumstances such as a break in the skin of the recipient increase the probability of transmission of multiple viruses. —AvB

It is also not always possible to find enough cells in the ejaculates of HIV-infected men that would allow for isolation and sequencing of the cell-associated HIV in the donors, says Ron Swanstrom, director of the University of North Carolina Center for AIDS Research. In fact, Swanstrom says, there are often so few cells that it seems questionable whether there are enough cells to mediate transmission. “We do find CD4⁺ T cells, but not at significant levels that would suggest enough infected cells to contribute to transmission,” he says. Anderson says she has also found that HIV-infected cells such as

CD4⁺ T cells are not always detectable in semen from HIV-infected men, but adds that there is great variation. For example, many more HIV target cells can often be found in the semen of HIV-infected individuals with inflammation in the genital tract from other sexually transmitted diseases.

For all these reasons, the studies of the role of cell-associated virus in HIV transmission in semen have so far involved too few transmission pairs to yield meaningful results, Mullins says. “We really need to do large-scale analyses and do them carefully,” he says.

In what may be the largest effort so far to analyze semen in HIV transmission, Lingappa and colleagues, in collaboration with Mullins and Lisa Frenkel at the University of Washington, hope to analyze semen samples that were taken within six months of infection from HIV-infected men in up to 20 heterosexual transmission pairs. In all of the samples, sequencing has already confirmed that transmission occurred between the partners. The samples come from a study of 3,408 discordant couples from

Africa that showed that acyclovir treatment of the HIV-infected partner did not reduce HIV transmission (*N. Engl. J. Med.* 362, 427, 2010). While the timing between transmission and sampling in the study won’t be very close in most cases, Mullins hopes that the larger number of cases compared with previous studies will provide new insights. “We might be able to identify consistencies not evident previously,” he says.

Mother-to-child transmission

HIV-infected cells also seem to play a role in MTCT of HIV. A 2004 study of almost 300 antiretroviral (ARV) untreated mother-infant pairs in Africa led by Overbaugh showed that cell-associated virus levels in the mother were a more important predictor of transmission risk to the child than cell-free virus levels (*J. Infect. Dis.* 190, 1880, 2004). This finding suggests that the children might get infected by ingesting HIV-infected cells from the mother, Overbaugh says.

Aldrovandi and her colleagues are planning an even bigger study of this type, using samples from about 600 mother-child transmission pairs from the Zambia Exclusive Breastfeeding Study (*N. Engl. J. Med.* 359, 130, 2008). The researchers will correlate the levels of cell-free and cell-associated virus in the mothers’ breast milk with the transmission risk to the infants. One advantage is that the researchers know quite well when the infants were infected, Aldrovandi says. She and her colleagues may also try to determine HIV sequences from some of the mother-child transmission pairs to see more directly whether the cell-associated or the cell-free HIV caused the infection in the infants.

Overbaugh’s study used samples that were collected before mothers were advised to take ARVs during delivery or breastfeeding, which is now becoming more common practice because it has been shown to dramatically reduce rates of MTCT. However, Overbaugh and others have found that ARV treatment of the mother mostly reduces the mother’s free virus level, not the reservoir of HIV-infected cells in the breast milk (*AIDS* 22, 1475, 2008). That raises a question, Overbaugh says: If, as her 2004 study suggests, cell-associated virus plays an important role in MTCT, how is it possible that ARV therapy dramatically reduces the MTCT risk if it mostly reduces cell-free, but not cell-associated virus levels in the mothers? One possibility, she says, is that the ARVs are actually working as prophylaxis in the infant. Consistent with this, Overbaugh says, a recent study showed that infant ARV prophylaxis during breastfeeding can reduce MTCT even if the

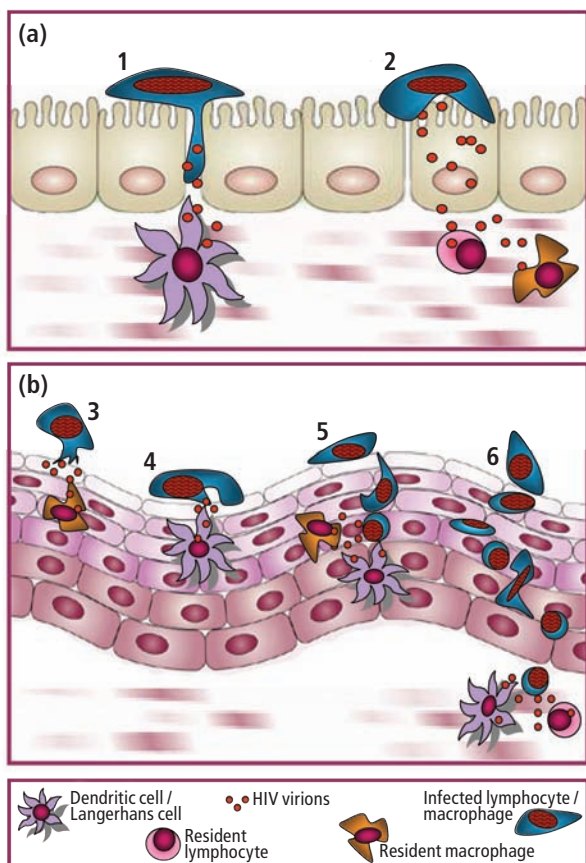


Figure 1. Potential mechanisms underlying cell-associated HIV transmission. (a) Columnar epithelium: (1) Infected cell migrates between epithelial cells to infect susceptible host cells. (2) HIV transcytosis through epithelial cells to infect susceptible target cells. (b) Stratified squamous epithelium: (3) Transfer of HIV from infected leukocyte to epithelial cell, which transfers virus to target cells through transcytosis or attraction via release of chemokines. (4) Direct cell-to-cell transfer of HIV from infected leukocyte to target cell via viral synapses. (5) Transepithelial migration of infected leukocyte to infect target cells within the epithelium. (6) Transepithelial migration of infected cell to infect target cells in the subepithelium or draining lymph nodes.

Originally published in *AIDS* 24, 163, 2010.

mother is not receiving treatment (*N. Engl. J. Med.* 362, 2271, 2010).

Cell-bound virus challenge models

While most challenge models in NHPs currently use cell-free challenge stocks, in a handful of studies researchers have tried to infect NHPs with cell-associated virus. Studies in female chimpanzees and cynomolgus macaques suggested that infection with cell-associated HIV or SIV respectively is possible, while an attempt to infect female rhesus macaques with SIV-infected cells failed (*AIDS Res. Hum. Retroviruses* 14 Suppl 1, S119, 1998).

But a study last year (*J. Infect. Dis.* 202, 337, 2010) that was led by Roger Le Grand, head of the division of immunovirology at the Institute for Emerging Diseases and Innovative Therapies at the Atomic Energy Commission in France, was an important advance towards the development of a challenge model for cell-associated transmission in macaques, says Anderson, who wrote a commentary on the study (*J. Infect. Dis.* 202, 333, 2010).

In the study, Le Grand and colleagues infected cynomolgus macaques with SIVmac251 and isolated SIV-infected CD4⁺ T cells and macrophages from their spleen at peak viremia. They washed off any free SIV and placed the infected cells onto the vaginal mucosa of uninfected cynomolgus macaques that had been treated with Depo-Provera to facilitate transmission by thinning the vaginal mucosa. Four of the five macaques challenged this way got infected after one challenge. In addition, when the researchers labeled the cells placed on the vaginal mucosa, they found the cells, as well as SIV, in distant lymph nodes as soon as 21 hours after challenge.

Still, it's hard to really prove that the macaques in this model got infected from the SIV-infected cells and not from free SIV particles, says Ronald Veazey of Tulane University. "SIV/HIV infected cells in fluids of an inoculum are not just quiescent, they are shedding virus like crazy," Veazey says. "So if you inoculate animals even with highly washed infected cells, it's still difficult to prove infection didn't occur due to shed virus from the cells instead of the cells themselves."

Le Grand says he addressed this issue by washing the SIV-infected cells several times, and by demonstrating that the supernatants from these washes, which should contain the free virus particles, did not infect the macaques. Still, he adds, it is possible that some SIV particles may have stayed attached to the cells, or that the cells may have infected the macaques by shedding virus close to the vaginal epithelium or

in contact with cells in the vaginal epithelium. "We are careful by saying that we transmit infection by cell-associated virus and not really by infected cells because we don't want to exclude the different mechanisms by which this can occur," Le Grand says.

Nevertheless, he says, the fact that labeled cells were observed in lymph nodes far from the vaginal mucosa just 21 hours after the challenge suggests that the cells did migrate away from the vaginal mucosa. "If this mechanism is true, it would be a very efficient mechanism to transmit infection," Le Grand says, and strategies designed to block the free viral particles, such as antibodies, may not work against these infected cells. He says he wants to use the challenge model to test if microbicides that have so far shown some efficacy to protect against cell-free challenge can also protect against cell-associated challenge.

The model is an important advance, Anderson says, in part because the dose of cellular HIV copies needed to infect half of the female macaques is similar to the number she and others have found to be present in human ejaculates. This is in contrast to the super-physiological doses needed to achieve infection with cell-free HIV.

However, the reception by the field to the study has been "flat," Anderson says. Indeed, some researchers are skeptical. Ashley Haase at the University of Minnesota believes that the cell-associated NHP model still has a long way to go until it can be used as a challenge model. He says that while there is a large body of information on what has been learned from the high-dose cell-free model of SIV infection of rhesus macaques, the literature on cell-associated infections is "scanty."

Many cell-associated infection studies so far, he adds, are also limited by confounding factors that could affect the results, such as the use of Depo-Provera by Le Grand and colleagues to thin the vaginal mucosa. Haase also notes that he and Eva Rakasz of the University of Wisconsin-Madison used macaques with chemically induced ulcers in the vaginal mucosa when they showed, similar to Le Grand, that SIV-infected cells placed on the vaginal mucosa are disseminated throughout the body (*J. Virol.* 82, 4154, 2008).

Anderson, for her part, is not deterred. "I don't intend to give up on our cell-associated HIV transmission research anytime soon," she says. "My colleagues and I that study cell-associated HIV transmission believe that the sidelining of this research area by leaders in the HIV prevention field may have delayed the development of highly effective HIV microbicides and vaccines." ■

Chemistry Lab

What happens when HIV researchers fall in love at work? Four tales about science and romance.

For nearly 30 years, the study of HIV/AIDS has attracted some of the brightest minds in science, and it's not too surprising that a number of those researchers have linked up outside the lab. How these research couples have been able to successfully intermingle science and love is shown in the following vignettes, which for very unscientific reasons IAVI Report chose to feature in the same month as Valentine's Day. Like many couples, these scientific duos struggle to find balance between work and home. When they're not competing for grants, authoring papers, or teaching, some are conducting research and running laboratories in places far from home. There was no shortage of couples working on HIV research to choose from, but ultimately we tried to highlight a few who work in both clinical and basic science and whose work overlaps substantially.

**By Kristen Jill Kresge,
Regina McEnery, and
Andreas von Bubnoff**

RON GRAY AND MARIA WAWER

Not every married couple can claim to have one of the largest collections of foreskins on the planet.

Ron Gray and Maria Wawer amassed these specimens studying whether adult male circumcision could reduce HIV infection rates. And while it might be tempting to crack a joke or two, there is nothing funny about their purpose: These samples could prove uniquely valuable in examining the role of HIV transmission at the mucosal level.

Ron is a professor in the Department of Population and Family Planning, and Maria is a professor in the Department of Population, Family and Reproductive Health, both at the Johns Hopkins Bloomberg School of Public Health. Several years ago, their Phase III study, along with two other studies by other research groups, showed at least a 60% reduction in HIV incidence among adult heterosexual men in Africa who underwent circumcision. The couple has been advocating ever since for implementation of this surgical procedure as an HIV prevention strategy.

Ron and Maria have been studying HIV/AIDS, side-by-side, for more than 20 years. Their research is conducted largely through the Rakai Health Sciences Center, a Uganda-based program that began as the Rakai Project to study the magnitude and dynamics of HIV, and has developed into a multi-institutional collaboration employing more than 400 people. Maria helped launch the Rakai Project in 1987

when she was an assistant clinical professor at Columbia University. Three years later, Ron, a trained epidemiologist, shifted his research to HIV as well.

Theirs is a close-knit arrangement that both acknowledge might not suit everyone. They have adjoining offices at Hopkins and fly back and forth together to Africa. They work on almost all their projects together and share the same staff. They author the same papers and present at the same conferences. Maria notes that their “skill sets” are extremely complimentary and that they have become a really great team. “We are never lonely. Researchers can sometimes get lonely,” she says.

Outside of work, they spend a lot of their free time at the movies—seeing about 200 films a year, many foreign—and cater to their three rescued dogs. Ron’s two children from a previous marriage are grown and Ron recently became a grandfather.

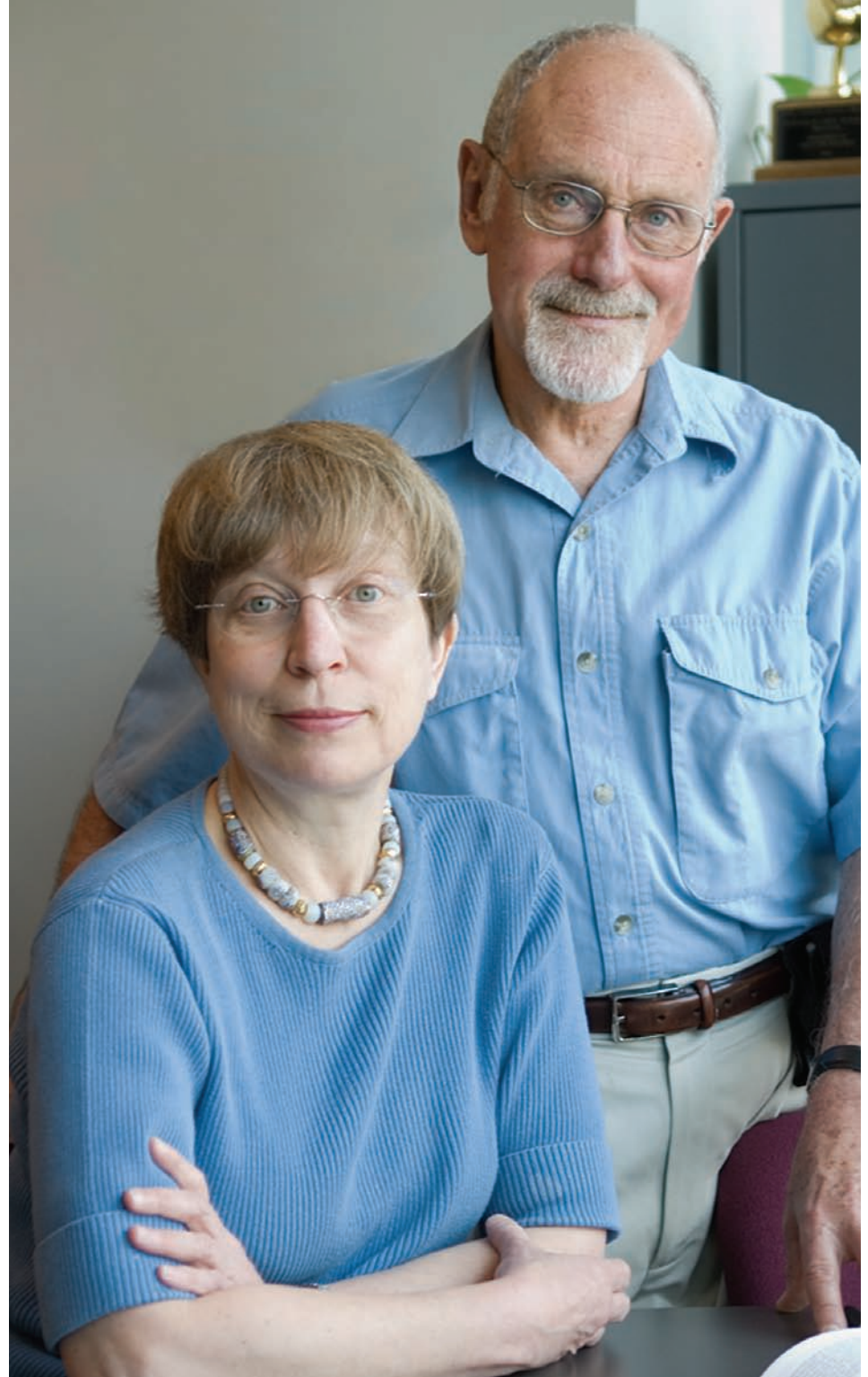
So does their working relationship ever feel too intense? “I would say one of the greatest disadvantages of working together is that every three days you want to kill each other,” Maria jokes. “On the plus side,” says Ron, “all our arguments are not about personal matters, but about science.”

Sometimes their disagreements are very public. One time, Ron and Maria were invited to an international AIDS conference to debate each other on a topic that neither could recall. But they both remembered how high the stakes were. “I said if you win there is no sex for six months,” says Maria.

Guess who prevailed, Ron says, laughing, though he insisted it was on the merits of her argument.

Ron met Maria in the early 1980s, when she dropped by his office at Hopkins. “I was completely bowled over by this red-hot lady,” he recalls. “She was literally radiating heat.” Indeed, Maria was battling a high fever and bug bites that had festered into tropical ulcers following a recent trip to Africa.

But for Ron, that wasn’t the only kind of heat Maria was radiating. Within six



months, Ron and Maria were involved romantically. They lived together for about a decade until one night, at the urging of a friend and after a night on the town, Ron stuck a plastic flower in his mouth, knelt down on the sidewalk, and said what Maria heard as, “Will you marry me?”

“Till this day, he insists he said, ‘Will you carry me,’” she says.

DAVE AND SHELBY O'CONNOR

Most people probably wouldn't think a monster truck rally is very romantic. But for Dave and Shelby O'Connor, it did the trick. "I think we started dating after that," Shelby remembers. "No one can resist the charm of a good monster truck rally," says Dave, jokingly.

The rally took place in the late 1990s in Urbana-Champaign, where the two had become friends as undergraduate students. Shelby later moved to join Dave as a graduate student at the University of Wisconsin-Madison. In 2001, Dave proposed to Shelby on the top of a mountain in Canada. "She asked if I was kidding," he says. "I said no and she said, 'Oh, in that case, sure!' And that was that." They were married in 2002.

Dave is now an associate professor at the department of Pathology and Laboratory Medicine at the University of Wisconsin-Madison. Shelby works as an associate scientist in his lab, with 70% of her salary and time paid by her own grant, which also pays for her own technician. "I do some stuff with him and for him and some stuff is part of my own grant," she says, adding that she might become even more independent in the future. "I think that moving forward I do need to develop some related but independent projects so that we will have different paths."

One of their most important shared projects is their two and a half year old son, Eli. One thing that prepared them for



raising a child was to have pets, Dave says. First, they got a lovebird named Noodles, and then a dog named Triscuit. "Each one of them was a trial," he says. "The bird was to show that we can be responsible for some other living entity and the dog was a trial in structured responsibility."

Full-time research schedules and a small child keep them very busy, but they say they have found a way to manage it all. They take Eli to daycare in the morning, pick him up at around 4:30 pm, and then go home to spend time with their son until he goes to bed. "We try very, very hard to have dinner together," Dave says. To make up for the lost time, they often work some more in the evening, in what Dave calls their second shift. "One of the tradeoffs for trying to keep from 4:30 until about 7:30 open for the family is that we pretty much need to recover some additional work time most nights. That's just a tradeoff we are happy to make."

As a couple, they enjoy sports and outdoor pursuits. Now that they have a small child, they don't have as much time to work out together as they used to, Shelby says, but twice a week they have a

"lunchtime date" to go swimming. Shelby says the five-minute drive to the gym is when she has some of the best discussions with Dave about work.

One piece of advice they have for couples who try to manage research with a family is balance. "We both are very committed to working on HIV because it's a terrible global problem," Dave says, "but we have [to] make sure it's balanced against the priorities [of] maintaining a family and other relationships."

One perk of being married to a scientist, Dave says, is that Shelby is a "bad idea filter." When she doesn't like some of his ideas, she just rolls her eyes. When Dave said he was going to make a video to announce the 2008 nonhuman primate meeting in Puerto Rico, starring him and another scientist in the lab singing "Kokomo" by the Beach Boys while playing the guitar and wearing latex gloves and lab coats, Shelby was skeptical. "I think I did roll my eyes at that and I thought, what are you crazy?" she says. "But it did turn out to be kind of funny in the end." Judge for yourself: <http://labs.pathology.wisc.edu/oconnor/multimedia/videos.html>.

||| SUSAN ALLEN AND ERIC HUNTER

It can be challenging for an epidemiologist and a molecular immunologist to find common ground, particularly when one is half-a-world away in Africa, but Emory University AIDS researchers Susan Allen and Eric Hunter managed to do just that, and found love in the process.

Susan gets credit for sparking the collaboration. Both get credit for making it work. The couple met in 1996 at the University of Alabama-Birmingham, where Eric headed the Center for AIDS Research (CFAR) and Susan was a newly recruited epidemiologist from the University of California, San Francisco. Susan invited Eric to visit her clinical sites in Africa so CFAR could see the work that was being done there.

The visit put the epidemic in perspective for Eric, who until then had never been to sub-Saharan Africa. They also found they were quite compatible. “We discovered it was a lot of fun to work together,” Eric recalls.

“Before we met, Eric had not even been working on viruses from Africa,” says Susan. “I think in a way by marrying him I kind of pulled him into this realm and it worked out really well for everybody.”

The most prominent example of their work collaboration involved data from Eric’s lab suggesting that in most cases of heterosexual HIV transmission just one transmitted virus variant is responsible for establishing a productive infection in the recipient, suggesting that there is a genetic bottleneck that limits the degree of variation as the virus is transmitted. Eric, a professor of pathology and laboratory medicine at Emory, was able to reach this conclusion analyzing samples from serodiscordant couples from the Rwanda Zambia HIV Research Group (RZHRG) that Susan founded in Rwanda in 1986 when she was still in California. Susan, a professor of global health at the Rollins School of Public Health at Emory, remains the driving force behind the RZHRG, which tracks the longest-running and

largest cohort of HIV serodiscordant couples in the world.

Eric and Susan face many of the same challenges as other working couples, with their situation made even more complicated by the fact that they are separated for several weeks at a stretch. They also both have children from previous marriages. When they met, Susan’s sons were two and three and Eric’s daughters were 11 and 13. “As parents we couldn’t be gone from home so we made a pact that we wouldn’t be away from the kids for more than a week with both of us gone. We held to that,” says Susan.

“I think if you ask any scientist, the hardest balance is home and work,” adds Eric. “Research is not a nine to five job. It is quite consuming in terms of what you are thinking about and so trying to make that balance is one of the hardest things for me. We do have times when we go out to dinner and we say to each other we are not going to talk work tonight. The hardest thing is to not constantly bring your work into everything you do.”

But if you do have to bring your work home with you, Susan jokes that you can charge for pillow talk the same way lawyers bill for hours, only to add that it never really feels like work. “Of course when we are talking about this stuff it’s because we love it,” she says.



IV. SALIM AND QUARRAISHA ABDOOL KARIM

Circumstances were not in their favor when Salim and Quarraisha Abdool Karim connected at a party in 1987. The party was being thrown in Salim's honor—five days later he was moving from the University of KwaZulu-Natal in Durban, South Africa, to New York City to study epidemiology at Columbia University. He and Quarraisha managed to squeeze in a few dates in the short time before his departure and there was instant chemistry. “We sort of knew each other for about five days and decided it was time to get married,” Salim remembers.

They kept in touch through letters, and then six months later they were married in South Africa. The wedding, a 600-person affair, foreshadowed the couple's ability to organize large-scale events that have come to be some of the hallmarks of their careers together as co-principal investigators of HIV prevention trials.

After the wedding, Quarraisha joined Salim in New York City and also studied epidemiology at Columbia. Ironically, it was here and not in their home country, which is now the epicenter of the pandemic, that their focus turned to HIV. “When we came back to South Africa in 1989, we basically established ourselves as researchers ready to tackle HIV full steam,” says Salim.

By 1990, they published their first research paper together. At that time South Africa was still under apartheid and the couple was actively involved in anti-apartheid activism in addition to their research. Once apartheid was lifted, South African researchers could at last apply for international research grants. In 1997, Salim applied for and received his first research grant from the Wellcome Trust in the UK. Soon after, he received a grant from the US National Institutes of Health.

Around that same time Salim and Quarraisha became co-investigators of a microbicide trial to see if a new gel



formulation of the spermicide nonoxynol-9 (N-9) could prevent HIV infection in a cohort of female sex workers. The trial's data and safety monitoring board (DSMB) stopped the trial early, but the investigators were still blinded and didn't know which arm had the higher HIV incidence. “I was so excited to hear that the DSMB stopped the trial,” Salim recalls, having assumed at the time that the microbicide had worked. It wasn't until about eight months later that the investigators learned that the HIV incidence was actually substantially higher in the N-9 group. “I was shocked,” says Salim. “We were organizing for the AIDS conference in Durban and we thought this microbicide trial was going to be the highlight only to learn that we were going to give the worst news of the conference.”

This spectacular failure didn't deter the husband and wife team. Salim and Quarraisha went on to conduct other microbicide trials together, and last year their determination finally paid off. Although they could not deliver good news in Durban in 2000, they stole the show ten years later at the International AIDS Conference in Vienna, announcing that a Phase IIb trial they co-led in South Africa had shown that a vaginal

microbicide gel containing 1% of the antiretroviral tenofovir reduced HIV incidence by a statistically significant 39%. The data, published in *Science*, was greeted with unbridled optimism. “It was just so exciting to be involved in it,” the couple recalls.

Even their three children—an 18-year-old daughter who studies law at the University of Cape Town, a 15-year-old daughter who is in high school, and a 12-year-old son in primary school—shared in the excitement. Although they both keep hectic travel schedules, they have a rule not to travel together so that one of them is always home with the children. Vienna was an exception. Sundays are reserved for family time and usually involve long walks or bicycle rides.

The biggest advantage they see in working together is in knowing how the other thinks, which isn't to say they always agree.

In her spare time, Quarraisha enjoys reading, either alone or with the children. She discloses that Salim's down time often involves a television. “Salim is not telling you about his passion for watching sports,” Quarraisha says. “I like cricket in particular,” he admits, “and no one wants to join me for five days of cricket watching.” ■

More than *Trial and Error*

Veteran vaccine developer Stanley Plotkin reminds researchers that not all vaccines were developed without any notion of how they worked

“Time present and time past are both perhaps present in time future.” —T.S. Eliot



Stanley A. Plotkin, MD

The writer, an emeritus professor of the University of Pennsylvania and the Wistar Institute, developed the rubella vaccine that is used throughout the world, is co-developer of the pentavalent rotavirus vaccine, and has worked extensively on the development and application of other vaccines including anthrax, oral polio, rabies, varicella, and cytomegalovirus. He is a consultant to all major vaccine manufacturers.

AS NEW STRATEGIES FOR VACCINE development proliferate, mainly based on genetic engineering, and as systems biology comes into its own, it has become cliché to say that prior vaccines were developed empirically, without any idea of the mechanisms leading to effective immunogens or the ways to produce them. Perhaps a member of the older generation may be forgiven for pointing out that this is a canard with respect to any vaccine developed since the mid-20th century, and is insulting to a large group of living and dead researchers.

It is true that Jenner had no clear idea of how vaccination protects when he used vaccinia virus to prevent smallpox. Rather, he developed this approach based on the observation that milkmaids were protected against the disease by prior exposure to cowpox, and on the analogy of vaccination to variolation, the centuries-old practice of minimizing the severity of a later smallpox infection by deliberate infection with dried smallpox scabs in order to provide immunity during subsequent exposure to natural smallpox. It is also true that Pasteur’s discovery of attenuation of the chicken cholera bacillus some 80 years later was serendipitous.

Nevertheless, Pasteur applied attenuation techniques to other organisms including the anthrax bacillus, and, after his famous experiment at Pouilly-le-Fort showed protection by the anthrax vaccine, he stated that it was now possible to generalize his methods and to make vaccines at will. Moreover, by the end of the 19th century, Salmon and Smith in the US and Roux and Chamberland in France developed methods to kill bacteria with the expressed purpose of rendering them harmless while guarding their immunoge-

Hope for the future does not require denigration of the past.

– Stanley Plotkin

nicity. “Immunity,” they wrote, “is the result of the exposure of...the animal body to the chemical products of the growth of specific microbes which constitute the virus of contagious fevers.”¹

The discovery of diphtheria and tetanus toxins by Behring and Kitasato, also at the end of the 19th century, confirmed that substances secreted by bacteria could cause disease. In addition, they demonstrated that injection of those substances elicited neutralizing factors in blood. Early in the 20th century, Paul Ehrlich’s idea of antibodies as substances generated by the host in response to foreign antigens became accepted, and when Ramon inactivated diphtheria and tetanus toxins by formalin to produce toxoids, his purpose was to induce antibodies to the toxins without causing adverse reactions.

During the first half of the 20th century, two major vaccines were developed, BCG [Bacillus Calmette-Guérin] and yellow fever. In both cases, the idea was that passage of a pathogen in bacteriological media or in an unnatural host would weaken its virulence for the natural host, as had been demonstrated by Pasteur for rabies years before. Success was achieved by Calmette and Guérin, who developed the BCG vaccine, and by Max Theiler, who developed the yellow fever vaccine, although it should be noted that Theiler had several competitors who were also passaging yellow fever virus in animals. All were pursuing the idea that selection by passage in a foreign host or *in vitro* would favor attenuation. Although they and subsequent researchers knew nothing of genetic engineering, they did know that their methods were selecting for attenuated variants.

The discovery that viruses could be grown in cell culture allowed expanding the method of attenuation by serial passage in cell culture. Of the two polio vaccines that launched the mid-20th century explosion of vaccines, the oral vaccines of Koprowski and Sabin followed precisely that idea. They selected populations of poliovirus cloned by plaquing in cell culture and tested each one for monkey neurovirulence. Similarly, measles and mumps vaccines were created by passage in cell culture, with attenuation measured in humans. During the fiercely competitive activity to develop avirulent strains of polio, it was found that passage at suboptimal growth temperatures allowed more rapid

attenuation, a principle followed in the development of rubella vaccine. Also, varicella vaccine could be created because passage in guinea pig instead of human cells exerted a profound selection for attenuation.

Meanwhile, Salk’s inactivated polio vaccine followed directly his work on inactivated influenza vaccine, the goal of both being to produce antibodies. In fact, the success of the polio vaccine was foretold by the prior demonstration that polio could be prevented by serum antibodies contained in pooled gamma globulin.

Development of the vaccines that have followed was also predominantly guided by the principle that the induction of antibodies either in the serum or on the mucosa is the way to prevent infection, as repeatedly demonstrated by prior experimentation. This principle extends to the vaccines recently produced by genetic engineering: hepatitis B and human papillomavirus. Rotavirus vaccines were developed by serial cell culture passage or by reassortment of RNA segments of the viral genome to induce limited replication in the intestine with the aim of inducing mucosal antibody and local cellular responses similar to those after natural infection. In contrast, the zoster vaccine induces T-cell responses that prevent the reactivation of varicella virus latent in neurons, following another established principle that once infection takes place, T-cell responses control replication.

My desire here is not to deny the hope we all have that future vaccine development will be informed by new strategies, including the identification by systems biology of genes whose products predict protection. However, hope for the future does not require denigration of the past. The main difficulty we face in the case of vaccination against HIV is our inability to identify naturally protective immune responses, particularly on the mucosa. However, studies in non-human primates and the recent moderately successful RV144 trial suggest that as in other diseases, antibody of the right specificity and functionality prevents infection, whereas cellular immunity controls replication.²

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1. Salmon DE, Smith T. On a new method of producing immunity from contagious diseases. *Amer. Vet. Rev.* 10: 63-67, 1886
 2. Plotkin SA. Correlates of protection induced by vaccination. *Clin. Vaccine Immunol.* 17: 1055-65, 2010.

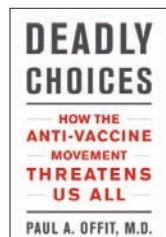
Fearing Vaccines

More than Disease

Two books examine the roots and repercussions of a growing anti-vaccine movement

By Kristen Jill Kresge

“WHEN VACCINES WORK, nothing happens,” says Paul Offit, chief of the division of infectious diseases and director of the Vaccine Education Center at the Children’s Hospital of Philadelphia. And so it isn’t terribly surprising that the absence of several infectious diseases for which there are highly effective vaccines goes largely unnoticed. But now, some of these preventable diseases, which were all but eradicated in many wealthy countries, are on the rise.



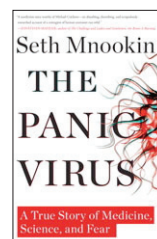
DEADLY CHOICES
How the Anti-Vaccine Movement Threatens Us All
 By Paul A. Offit, M.D.
 288 pages. Basic Books.

As both Offit, in his new book *Deadly Choices, How the Anti-Vaccine Movement Threatens Us All*, and Seth Mnookin, in his new book *The Panic Virus: A True Story of Medicine, Science, and Fear*, explain, the resurgence of vaccine-preventable diseases stems from a growing number of parents who are choosing not to vaccinate their children. According to a study published in the *Journal of the American Medical Association* in 2006, the percentage of unvaccinated children in the US has more than doubled since 1991.

This trend has dire consequences, extending far beyond the US. “As more and more people have chosen not to vaccinate, herd immunity has broken down,” writes Offit. Mnookin cites several alarming statistics: in 2009, there were more than 13,000 cases of pertussis or whooping cough in Australia, the highest number ever recorded in that nation’s history. In Great Britain, the number of cases of measles has increased more than a thousand-fold since 2000. And in 2010, an outbreak of pertussis in California was so

serious (public health authorities have reported nearly 9,000 confirmed, probable, or suspected cases and 10 deaths since Jan.1, 2010) that it actually led some countries to warn their citizens about the possible dangers of travel to that area of the state.

Before the 1940s, pertussis was one of the leading causes of infant mortality in the world. After the vaccine was introduced, the number of deaths from this disease in industrialized countries dropped by more than 90%. Offit begins his analysis of the anti-vaccine movement by asking, “How did we get here? How did we come to believe that vaccines, rather than saving our lives, are something to fear?”



THE PANIC VIRUS
A True Story of Medicine, Science, and Fear
 By Seth Mnookin
 448 pages. Simon & Schuster.

The roots of the anti-vaccine movement are indeed complex, but there are several individuals and institutions that both Offit and Mnookin accuse of perpetrating anti-vaccine rhetoric, and as they convincingly classify it, fallacy. Both authors pin much of the blame on the media, whose slapdash reporting on issues of vaccine safety and reliance on controversy to sell papers or boost ratings has helped fuel the movement against vaccines. “It’s the media that provided—and continues to provide—the fuel for this particular fire,” writes Mnookin, who is a contributing editor at *Vanity Fair*. Offit and Mnookin both pinpoint the birth of the modern American anti-vaccine movement to a specific day. It was April 19, 1982, when a television station in Washington, D.C. aired a one-hour show titled “DPT: Vaccine Roulette.” The show focused on claims of brain damage, mental retardation, and neurological damage that

resulted from the pertussis vaccine, one of the three components of the DPT vaccine (the others being diphtheria and tetanus). Mnookin acknowledges that DPT is a reactogenic vaccine and that doctors had been aware that it could cause seizures, high fevers, and fainting. But “Vaccine Roulette” depicted numerous stories of children who experienced permanent neurological and developmental damage after DPT vaccination. Offit says this show inspired multiple news outlets across the country to write about the dangers of the pertussis vaccine and caused thousands of parents to stop vaccinating their children. The airing of “Vaccine Roulette” had another profound effect—it inspired so many lawsuits against vaccine makers for vaccine-related injuries that it led the US Congress to pass the National Childhood Vaccine Injury Act that protected the vaccine manufacturers by shifting the burden of litigation from the companies to the government.

In her book, *A Shot in the Dark: Why the P in the DPT Vaccination May Be Hazardous to Your Child’s Health*, published nearly a decade after “Vaccine Roulette” first aired, Barbara Loe Fisher was the first to suggest specifically that vaccines might be linked to autism. Then in 1998, Andrew Wakefield, a surgeon with the Royal Free Hospital in London (Wakefield was stripped of his medical license in 2010), was the lead author on a now infamous study in *The Lancet* suggesting a possible link between gastrointestinal disease and the onset of behavioral disorders, including autism, in children following receipt of the measles, mumps, rubella (MMR) vaccine. This study, which the journal retracted in 2010, was once again the subject of scrutiny when investigative journalist Brian Deer published a series of articles in the *British Medical Journal* in January 2011 documenting how Wakefield and colleagues had misrepresented the medical histories of most of the 12 children on whom the study was based, and even more damning, that Wakefield profited from this “elaborate fraud.” As both Offit and Mnookin relate, this study resulted in plummeting MMR vaccination rates in the UK.

Even as studies showing there was no connection between autism and MMR vaccination mounted, the anti-vaccination campaign in the US gained momentum. This time there was another culprit, thimerosal.

In 1999, the US Centers for Disease Control and Prevention and the American Academy of

Pediatrics called for the removal of a mercury-based preservative called thimerosal from vaccines because they worried that children might be receiving too much mercury. This fueled another vaccine-related scare, taken up by notable public figures like Robert F. Kennedy, Jr. and the actress Jenny McCarthy, and caused more parents to shun vaccines because of suggestions that thimerosal was also linked to autism. In 2001, thimerosal was removed from all vaccines, and then in 2004, an independent committee appointed by the prestigious US Institute of Medicine concluded “the body of epidemiological evidence favors rejection of a causal relationship between the MMR vaccine and autism. The committee also concludes that the body of epidemiological evidence favors rejection of a causal relationship between thimerosal-containing vaccines and autism.” This report, coupled with the dismissal of several lawsuits as part of the Omnibus Autism Proceeding discussed in detail in both books, did little overall to quell the anti-vaccine movement.

So what will reverse the tide? “We’ve reached a tipping point,” writes Offit. “Children are suffering and dying because some parents are more frightened by vaccines than by the diseases they prevent. It’s time to put an end to this.” Offit proposes a few possible solutions, the least appealing of which is for the incidence of childhood deaths from vaccine-preventable diseases to become so high that parents once again recognize the value of vaccines. Another is eliminating the religious and philosophical exemptions that allow parents in certain states to admit their children to school without being vaccinated. Offit also urges doctors to be more proactive in explaining to parents the repercussions of foregoing vaccinations. But in the end, Offit recognizes that despite all the scientific evidence, this is really an emotional battle. Offit therefore appeals to parents to consider the greater immunological good. “When you choose for your child not to get a vaccine it’s not a choice that you’re making for yourself alone,” says Offit. “You’re making that choice for other people who may be too young to be vaccinated or who are getting chemotherapy for their cancer. They depend upon those around them to be vaccinated, and if they are not, then these are the people who are going to most likely suffer and die from diseases.” He certainly doesn’t have to convince Mnookin, who became a new father just as he was completing research for his book. ■

 Listen to a podcast of Paul Offit discussing the anti-vaccine movement on our website, www.iavireport.org

Vaccine BRIEFS

After Long Career, Peggy Johnston Retires from Post at NIAID



PEGGY JOHNSTON, WHO SERVED AS director of the vaccine research program at the Division of AIDS (DAIDS) at the US National Institute of Allergy and Infectious Diseases (NIAID) for the past 12 years, retired in December after a 30-year career that spanned both the public and private sectors, and in the last 15 years focused almost exclusively on the search for an AIDS vaccine.

Johnston, who just turned 60, joined NIAID in 1987 as a program officer. “I sort of think I grew up at NIAID,” she noted. In 1993, she was named deputy director of DAIDS. She left three years later to become IAVI’s founding scientific director and first senior vice president for scientific affairs. Johnston returned to NIAID in 1998 to serve as director of the DAIDS Vaccine and Prevention Research Program (now the Vaccine Research Program), managing a US\$351 million research portfolio by 2010.

Johnston helped see through the 16,000-person RV144 trial in Thailand that was launched under a cloud of controversy, but ultimately showed the first evidence of vaccine-induced protection against HIV (see *Raft of Results Energizes Researchers*, *IAVI Report*, Sep.-Oct. 2009).

The results of the RV144 trial represented an emotional high for the normally stoic Johnston, who uncharacteristically teared up when the results were first shared with investigators in 2009. Two years later, the field is forging ahead with an array of post-RV144 studies, in addition to pursuing other promising avenues of research, but Johnston says she doesn’t feel the need to stay. “I’ve never been the kind of person who had to be there at the end,” she says. Johnston believes the goal of developing an AIDS vaccine is reachable. “I’m thinking we’ll get there and I’ll be alive to witness it.”

Nelson Michael, director of the US Military HIV Research Program, one of the collaborators of the RV144 trial, said there were major operational issues with launching the study, and as a result it took twice as long to enroll participants in the trial. “Peggy was a rock during those times,” Michael recalls. “It was really important to have someone with her gravitas, and my respect for her, which was always high, grew enormously. We were kind of in the foxhole together.”

While she has left her current position, Johnston will still be assisting NIAID in the restructuring of its HIV/AIDS clinical trials networks. She and her partner, who directs The Heart Truth campaign and its Red Dress Project to inspire women to reduce their risk for heart disease, plan to stay in the Washington, D.C. area as they develop plans to move to the Pacific Northwest. —*Regina McEnerly*

New Commitments from Public and Private Sectors to Try to Eradicate Polio

EFFORTS TO ERADICATE POLIO got a much needed boost last month when government and private donors announced commitments totaling nearly US\$200 million to supply and deliver polio vaccines in a handful of countries where the disease is still endemic and to support disease surveillance.

UK Prime Minister David Cameron said his country intends to provide \$61 million to the Geneva-based Global Polio Eradication Initiative—roughly doubling the UK’s 2010 commitment. The Bill & Melinda Gates Foundation, which has already spent \$1.28 billion on polio eradication efforts, also announced that they will give an additional \$102 million to help stamp out the disease. In his annual letter describing the Foundation’s priorities for the coming year, Gates wrote, “We are so close, but we have to finish the last leg of the journey.”

This latest infusion of funding was announced in January at

the annual World Economic Forum in Davos, Switzerland, days after the Gates Foundation announced a \$100 million partnership with the Abu Dhabi government that will also devote about \$34 million to eradicating polio in Afghanistan and Pakistan, with the remaining \$66 million going toward the delivery of a vaccine for pneumococcal pneumonia.

Polio was once a global menace that struck fast and left many of its victims, such as US President Franklin Delano Roosevelt, paralyzed for life. The introduction of the Salk vaccine in 1955 and the Sabin vaccine in 1962 changed the course of the epidemic dramatically. Polio was largely eliminated in industrialized countries by the early 1980s, and an effort to vaccinate millions of people in poor countries has managed to rid the disease from all but a handful of countries. Last year, the World Health Organization (WHO), which oversees the Global Polio Eradica-

tion Initiative, reported fewer than 1,000 new polio cases, most of them in four endemic countries: Afghanistan, Pakistan, India, and Nigeria.

The quest to make polio the second disease in modern history to be eradicated—smallpox was the first—has been a long and expensive process. Public health authorities have already spent 22 years and \$6 billion trying to eliminate the virus.

D.A. Henderson says the smallpox eradication campaign that he led for the WHO took 14 years and cost roughly \$500 million in today's dollars. In contrast, he said, polio eradication is costing about \$1 billion a year. Still, David Heymann, previously the WHO representative of the director-general for polio eradication and now with the Epidemiology and Infectious Diseases Department at the London School of Hygiene and Tropical Medicine, believes it would be foolish to give up now. "It would be a shame to stop before you have finished the job," he says.

There are many challenges to eradicating polio in endemic countries. Massive floods last year in Pakistan exacerbated the spread of polio, which is transmitted in food and water contaminated with fecal matter. Political instability and lack of security

have prevented vaccination teams from reaching children in both Afghanistan and Pakistan, while a vaccine shortage, inadequate maps, and poor surveillance efforts left about 20% of Nigeria's children unvaccinated. High population density and poor sanitation are the main reasons polio persists in the states of Uttar Pradesh and Bihar in northern India.

The trivalent oral polio vaccine, known as tOPV, which contains weakened versions of three types of wild poliovirus, also has had lower efficacy (74%) in Uttar Pradesh and Bihar than in the remainder of India (85%)—possibly because the substandard living conditions make children from this region more prone to diarrheal diseases that can prevent the vaccine from working effectively. Also, the strains in the trivalent vaccine can interfere with each other, producing immunity to one strain but not another.

Oliver Rosenbauer, a spokesperson for the Global Polio Eradication Initiative, says last year there were only 42 cases of polio reported in these two Indian states, compared to 741 cases in 2009. Rosenbauer says this dramatic decline may be partly due to the introduction of a bivalent vaccine known as bOPV. —*Regina McEnergy*

Researchers and Advocates Consider Advantages of More Adaptive Clinical Trial Designs

AT THE AIDS VACCINE 2010 meeting last September, several researchers began discussing the benefits of employing so-called adaptive trial designs in the evaluation of HIV vaccine candidates (see *A Change of Tune, IAVI Report*, Sep.-Oct. 2010). Since then, this idea has been gaining momentum. On Feb. 10-11, 2011, the World Health Organization, the Joint United Nations Programme on HIV/AIDS, the Global HIV Vaccine Enterprise, IAVI, and the National Institute of Allergy and Infectious Diseases (NIAID) sponsored a meeting in New York City to discuss the current state of thinking on the use of adaptive clinical trial designs in HIV vaccine development. The meeting brought researchers, clinical trialists, regulators, and vaccine advocates, including several developing country representatives, together to discuss the opportunities and challenges associated with this approach.

Simply put, adaptive clinical trial designs are those that allow modifications to an ongoing trial based on interim data. These modifications can include everything from altering the number of volunteers to discontinuing an arm of the trial. All of these changes must be planned for and described in the trial protocol before it gets underway.

Such adaptive trial designs are not new—they are commonly used in other fields of research and have already been employed to some extent in HIV vaccine clinical trials. The biggest difference between the HIV vaccine trials conducted to date and some of the adaptive designs being considered now is

that the proposed designs would involve multiple arms testing different vaccine candidates, each compared to a single placebo arm, and would allow for investigators to discontinue one of the vaccine arms if it was underperforming compared to the other candidate. The goals of this type of adaptive clinical trial design are to evaluate more candidates, more quickly, with fewer resources. "We don't have a vaccine candidate that we want to trot into a Phase III trial," said Dean Follman, a statistician from NIAID.

Nearly 30 years after the first diagnosed case of AIDS, only a handful of vaccine efficacy trials have been conducted, a pace many researchers lament is much too slow. But one of the many unanswered questions on the use of multi-candidate adaptive clinical trial designs for HIV vaccine trials is whether there are enough eligible candidates to support this approach.

Other questions that were discussed during the two-day meeting included how and when to communicate trial alterations to the volunteers, under what circumstances the adaptive design is preferable to the standard two-arm trial, how to convince multiple vaccine manufacturers to consider having their vaccine candidate tested head-to-head against another, and what regulatory authorities would expect to approve such trial designs. These questions remain largely unanswered for now, but they will likely be discussed and debated more in the coming months. "It's all manageable but logistically difficult," said Pat Fast, chief medical officer at IAVI. —*Kristen Jill Kresge*

Research BRIEFS

Study Describes Onset of Broadly Neutralizing Antibody Responses in HIV-infected Individuals

BROADLY NEUTRALIZING ANTIBODY (bNAb) responses in HIV-infected people become detectable on average two and a half years after infection, and in some cases as early as one year after infection, according to a study led by Leo Stamatatos, director of the viral vaccine program at Seattle BioMed, a non-profit research institute (*PLoS Pathog.* 7, e1001251, 2011). The study is the first to show the onset of bNAb responses in such detail, Stamatatos says.

The study also for the first time shows that HIV-infected individuals who developed bNAb responses had more CD4⁺ T cells expressing the Programmed Death 1 marker, potentially suggesting that these people have more follicular CD4⁺ T helper (T_{FH}) cells in blood than people without such responses. These cells are believed to be important for affinity maturation of antibodies, which is thought to be important for the development of a bNAb response (see *Vaccines to Antibodies: Grow Up!*, IAVI Report, July-Aug. 2010).

The serum samples used in the study came from a Vanderbilt University cohort of 21 people and a cohort of 17 people from Massachusetts General Hospital in Boston, all infected with HIV clade B. The samples were isolated at several time points, from just months to almost seven years after infection. To qualify as broadly neutralizing, 20-fold or higher dilutions of the sera had to neutralize at least 75% of a panel of HIV isolates from clades A, B, and C. Based on this criterion, about a third (five of 17) of the individuals in the Boston cohort developed a bNAb response on average two and a half years after infection, Stamatatos says. A similar fraction of people with bNAb responses has also been

observed in previous studies, he adds.

Sera from a few individuals in both cohorts neutralized around 50% of the HIV isolates at about one year after infection. Data from a few individuals that were followed for up to seven years suggested that if bNAb responses had not developed by about three and a half years, they did not develop later.

“The finding that cross-reactive neutralizing activity can develop so early after infection is new,” says Hanneke Schuitemaker, a professor of virology at the Academic Medical Center in Amsterdam, who was not involved in the study. In agreement with the new study, her group has previously reported that bNAb responses broaden over time (*AIDS* 23, 2405, 2009).

Stamatatos and colleagues also found that the specificity of the early responses around two and a half years after infection is directed to only a few targets on HIV Env, primarily the CD4 binding site and targets on the native Env spike that are similar to the target of the bNAbs PG9 and PG16, which were isolated in 2009 by researchers at IAVI and The Scripps Research Institute (TSRI; see *Raft of Results Energizes Researchers*, IAVI Report, Sep.-Oct. 2009).

These targets are similar to some of the targets Laura Walker, a graduate student in Dennis Burton’s lab at TSRI, and colleagues identified in a recent study of the bNAb responses of 19 individuals, whose sera had among the broadest and most potent neutralizing activity from a cohort of 1,800 individuals infected for at least three years (see *Antibody Fever*, IAVI Report, Mar.-Apr. 2010; *PLoS Pathog.* 6, e1001028, 2010). The fact that the

bNAb responses in both studies are directed to just a few similar epitope targets suggests that the specificity of the earliest and chronic bNAb responses is similar, and that these epitopes are very immunogenic in the context of HIV infection, says Stamatatos.

Walker says this similarity in the epitope targets is interesting, but points to several differences between the two studies that might make them hard to compare. For one, the new study only included people infected with HIV clade B and not clade C, whereas her study looked at individuals from Africa, many of whom were infected with HIV clade C. In addition, she says, the two studies used different virus panels to test the neutralization breadth of the sera.

Walker adds that her study used a more stringent definition of neutralization breadth than the new study by Stamatatos and colleagues—her study used at least 100-fold serum dilutions. “We are more interested in the best donors [and in] the epitopes targeted by the antibodies in those donors,” Walker says. “It may turn out that less broad neutralizing sera have different specificities than sera from elite neutralizers.”

But Noah Sather, a staff scientist in Stamatatos’ lab and a coauthor of the new study, says the intent of the two studies is different. He says while Walker and colleagues determined which epitopes are used by the top 5% of neutralizers selected from a very large cohort of HIV-infected individuals, Stamatatos and colleagues analyzed all HIV-infected individuals in their cohorts to determine when breadth begins to develop in the general population of HIV-infected individuals. —*Andreas von Bubnoff*

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