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IAVI Report

The Publication on AIDS Vaccine Research

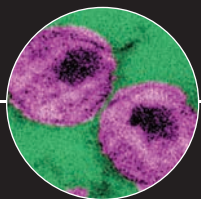
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Capsules from Keystone

FIRST IN A SERIES

Living History

Anthony Fauci on
The Quest for a Vaccine



“ We are living through one the most devastating pandemics ever to confront human civilization ”

EDITOR'S LETTER

I AM EXCITED TO INTRODUCE the inaugural installment of a new series, which we are calling “A Living History of AIDS Vaccine Research.” Its purpose is to provide perspective on historical moments in the quest for a vaccine, as well as insight into what lies ahead, as told by some of the leading researchers and policymakers in the field. We could not think of a better person to launch this series than Anthony Fauci, who has served as director of the National Institute of Allergy and Infectious Diseases (NIAID) for the past 25 years.

Fauci has been immersed in the AIDS pandemic ever since the first cases were described 28 years ago. He was involved in the development of the first antiretrovirals to treat HIV infection and has played a pivotal role in AIDS vaccine research and development—from his early decision not to fund the first Phase III trial of an AIDS vaccine candidate to establishing the Vaccine Research Center at NIAID. He's been one of the most vocal advocates and ardent supporters of the need for an AIDS vaccine and oversees a budget of US\$460 million dedicated to AIDS vaccine research and development, 30% of NIAID's overall HIV/AIDS budget. Whether Fauci is behind the podium at a conference or meeting with activists, he always eloquently captures both the current status of research and the human toll and devastation wrought by AIDS. He kicks off this series by explaining NIAID's role in vaccine discovery.

In addition to this article, a video podcast with Fauci is available to view or download on our website, www.iavireport.org. Since this is a new series, we would greatly appreciate your comments and suggestions, so please contact us at iavireport@iavi.org with any feedback. Additional chapters in the Living History series, which will each be accompanied by a video podcast, will focus on specific areas of research that beguile scientists and could offer clues that may help resolve some of the immunological mysteries of HIV.

At the recently held Keystone Symposia, which we devote substantial attention to in this issue, Françoise Barré-Sinoussi, a Nobel laureate for her role in the discovery of HIV, spoke of the importance of returning to basic science in AIDS vaccine research. As efforts shift in this direction, it is more important than ever to reflect on the past and gain insight into the path forward. We hope that the Living History series will do both.



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 24 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.

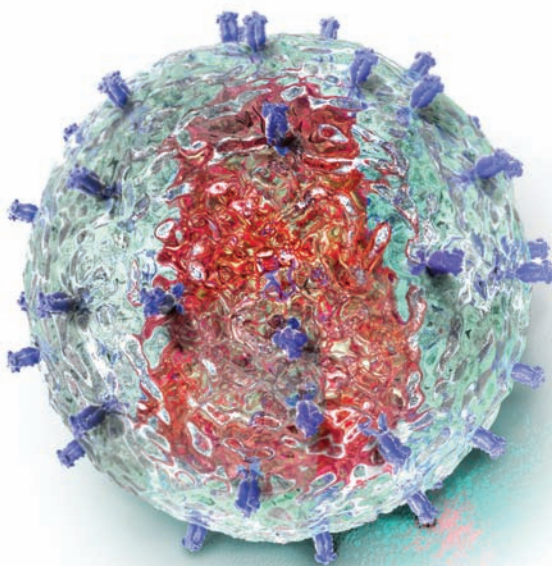
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[ON THE COVER]

Scanning electron micrograph of HIV-1 budding from cultured lymphocyte. Multiple round bumps on cell surface represent sites of assembly and budding of virions.

Image courtesy of the CDC / C. Goldsmith, P. Feorino, E.L. Palmer, W.R. McManus.

Capsules from KEYSTONE

A broad collection of updates at this year's joint symposia served to inform the development of future AIDS vaccine candidates

By Andreas von Bubnoff and Richard Jefferys

A LOT OF SNOW FELL the week of March 22-27 in Keystone, Colorado, accompanied by a flurry of updates on vaccine research and development by researchers who gathered for the joint Keystone Symposia on “HIV Immunobiology: From Infection to Immune Control” and “Prevention of HIV/AIDS.”

This year's meeting marked the 25th anniversary of Keystone Symposia's first meeting on HIV/AIDS, which was held in 1984, three years after the first HIV infections were described. The speakers at the conference's opening session left no doubt that 28 years after HIV emerged, there is still much to do. “We probably got rid of the iceberg, but under the water there is a mass of ice and that's the current AIDS epidemic,” said Didier Trono, of the Ecole Polytechnique Fédérale de Lausanne, in his introductory address. Nobel laureate Françoise Barré-Sinoussi from the Institut Pasteur said recent developments in the AIDS vaccine field show that “we have to come back to basic science.”

If the plethora of findings presented at this year's conference is any indication, researchers are already heeding her call. A broad collection of updates, ranging from imaging studies of viral transfer to results with vaccine candidates in ani-

mal models and clinical trials, all served to inform the development of future vaccine candidates.

Tracking transmission

Evidence has been accumulating to suggest that the majority of productive clinical HIV infections after heterosexual transmission can be traced back to a single transmitted founder virus (*Proc. Natl. Acad. Sci.* 105, 7552, 2008). This data, collected by George Shaw, a professor in the department of medicine at the University of Alabama at Birmingham, and others, is intriguing to vaccine researchers who have to deal with HIV's epic diversity (see *HIV Transmission: The Genetic Bottleneck*, IAVI Report, Nov.-Dec. 2008).

At Keystone, Shaw mentioned additional data that confirm and extend these observations. Of 171 heterosexual HIV transmissions studied, 81% can be traced back to one transmitted virus, he said, while in only 19%, more than one founder virus can be identified (*J. Virol.* 83, 3556, 2009).

The researchers also used 454 sequencing to analyze more fully an individual with evidence of a single transmitted founder virus—analyzing not just 30, but 600,000 *env* sequences in that person—and found the same result.

Shaw extended these studies to additional populations with different routes of transmission—men who have sex with men (MSM) and injection-drug users (IDUs). Sequence analyses of HIV in 49 MSMs showed that 40% of them had infections from more than one transmitted founder virus, which is double the percentage compared with heterosexual transmission, Shaw said. He presented data from one MSM whose infection could be traced back to six transmitted founder viruses. Preliminary results from IDUs suggest that 60% (three of the five studied) of infections stemmed from multiple founder viruses. One IDU Shaw evaluated was infected with at least nine transmitted founder viruses. “This is highly relevant for vaccine development because with a vaccine, you would ultimately like to prevent all of these transmissions,” said Shaw.

DNA/Ad5 against rigorous challenge

As findings by Shaw and others shed light on the transmitted virus in humans, researchers are starting to replicate this in animal models. David Watkins, professor at the department of pathology and laboratory medicine at the University of Wisconsin-Madison, collaborated with Shaw and Brandon Keele, then at the University of Alabama at Birmingham, to make a repeated low-dose rectal challenge with the swarm virus SIVsmE660 in macaques more similar to the situation in humans by titrating the challenge so only one to three virus variants would get across the mucosal barrier to cause infection.

The animals were vaccinated with a DNA prime and adenovirus serotype-5 (Ad5) boost regimen containing all SIVmac239 genes except for *env*. Intramuscular vaccination of the macaques with three DNA primes and a single Ad5 boost induced massive T-cell responses, the strongest of which were to Gag, targeting an average of 20 different epitopes of SIVmac239 (see *AIDS Vaccine Researchers STEP Up to the Challenge, IAVI Report, Sep.-Oct. 2008*).

Both the vaccinated and control animals became infected with the heterologous E660 after an average of four challenges. But the vaccinated animals had a lower average peak viral load than controls—32,000 copies/ml compared to 2.5 million in unvaccinated controls. Vaccinated animals also had a much lower average set-point viral load (201 copies/ml as compared to 77,000 for controls). This was the first time that a non-replicating T-cell vaccine showed such control of acute viral load, Watkins said.

The only other time this has been observed was with SIVmac239 Δ *nef*, he added. But while SIVmac239 Δ *nef* vaccinated animals are protected from intravenous (IV) challenge with the homologous SIVmac239 or SIVmac251, Watkins showed that they were not protected from IV E660 challenge. In some animals, SIVmac239 Δ *nef* recombined with E660 to give a much more pathogenic virus. “The stock of E660 was difficult to protect against even with our best vaccine after IV challenge,” Watkins concluded.

DNA prime doesn't pay

Dan Barouch, an associate professor of medicine at Beth Israel Deaconess Medical Center and Harvard Medical School, also reported results from a study in macaques testing a prime-boost regimen of DNA/Ad5 vaccines. Barouch's adenovirus vaccine, referred to as Ad5HVR48, is composed almost entirely (~98%) of Ad5, except for the hexon protein, which is swapped with the same protein from the less common Ad48 serotype.

The study involved 30 rhesus macaques, evenly divided into five groups, each receiving a different vaccination regimen (see table, right). Macaques with major histocompatibility complex (MHC) genes that have been associated with superior control of SIV replication (Mamu A*01, B*08, and B*17) were specifically excluded.

Six months after the last immunization, all animals were challenged intravenously with a high-dose of SIVmac251. Immediately prior to challenge, vaccine-induced SIV-specific T-cell responses were five-fold higher in the macaques that received the DNA prime/Ad5HVR48 boost compared to those that received only Ad5HVR48. But Barouch was surprised to find that post-challenge outcomes were not consistent with this immunogenicity data. Set-point viral loads were lowest in the animals that received only Ad5HVR48, averaging 4.4 logs, whereas in groups one and two, animals that received the DNA/Ad5HVR48 candidates had viral loads similar to those seen in the macaques that received placebo (5.2 logs in group one, 5.8 logs in group two, and 5.5 logs in the placebo group).

Barouch conducted a post-hoc exploratory analysis of the two Ad5HVR48 groups combined, compared to the two DNA/Ad5HVR48 groups combined. In this analysis, prime-boost recipients had higher viral loads (by around 0.75 logs) than those animals given only Ad5HVR48. Although this difference was statistically signifi-

Group	Vaccination Regimen
1	DNA/Ad5HVR48 (SIVmac251 Gag, Pol, Nef, Env)
2	DNA (SIVmac251 Gag, Pol, Nef, Env) + adjuvants encoding MIP-1 α and flt-3 ligand / Ad5HVR48 (SIVmac251 Gag, Pol, Nef, Env)
3	Ad5HVR48 (SIVmac251 Gag, Pol, Nef, Env)
4	Ad5HVR48 (SIVmac251 Gag, Pol, Nef)
5	Placebo

**[THE SHIFTING TREATMENT
LANDSCAPE: IMPLICATIONS FOR
VACCINE TRIALS]**

Scott Hammer, professor of medicine at Columbia University, reviewed the potential impact of antiretroviral therapy (ART) on future vaccine trials as part of the Prevention of HIV/AIDS symposium at this year's Keystone meeting, held March 22-27.

Hammer explained that with current vaccine candidates aiming to reduce post-infection viral load, early initiation of antiretroviral therapy (ART) has to be considered when designing studies, specifically evaluating how to censor viral load endpoints among participants who start ART. Although there is currently no consensus regarding initiation of treatment during acute infection, Hammer noted that many clinicians favor it, particularly because of studies indicating that gut T cells are rapidly lost during this period.

Treatment guidelines are also starting to shift toward earlier treatment, driven by data from recent studies, which found that earlier treatment significantly reduced the risk of illness and death (*N. Engl. J. Med.*, doi: 10.1056/NEJMoa0807252). This will have to be taken into account in vaccine trials that include time to initiation of ART as an endpoint, Hammer concluded.

For pre-exposure prophylaxis (PrEP), significant developments are close on the horizon. Results from the first efficacy trial may come this year and if PrEP is effective, investigators may need to consider whether it should be offered to vaccine trial participants. —RJ

cant, Barouch stressed that because this was an exploratory analysis, “the result must be viewed as hypothesis-generating rather than conclusive.” After 500 days of follow up, four of 12 animals in the DNA/Ad5HVR48 groups were alive, compared to 10 of 12 in the Ad5HVR48-only groups and one of six among placebo recipients.

In conclusion, Barouch speculated that the DNA/Ad5HVR48 was inferior because the DNA vaccine disproportionately increased CD4⁺ T-cell response to Env compared to the other antigens—prime-boost recipients showed four- to five-fold higher responses to Gag, Pol, and Nef, but 10-fold higher responses to Env. Barouch said one possible explanation is that Env-specific CD4⁺ T cells might have provided more targets for SIV, thereby counteracting the protective effect of vaccination seen in the Ad5HVR48 groups. A similar phenomenon was described before in an SIV challenge study involving a varicella zoster virus vector-based vaccine that only induced Env-specific CD4⁺ T-cell responses (*Proc. Natl. Acad. Sci.* 101, 13026, 2004).

Barouch's prime-boost regimen is similar to one developed at the Vaccine Research Center (VRC) at the National Institute of Allergy and Infectious Diseases (NIAID). The VRC's DNA/Ad5 prime-boost regimen is slated to undergo testing in a 1,200-person Phase II trial called HVTN 505, which is a smaller version of the originally proposed Phase IIb test-of-concept trial known as PAVE 100. Scott Hammer, the principle investigator of HVTN 505, stressed that Barouch's results could not be directly extrapolated to the VRC's DNA/Ad5 prime-boost regimen. There are differences—Barouch's vector has a different hexon protein and the VRC Ad5 vector also has additional genes deleted (E3 and E4), which reduces the expression of Ad5 proteins (*J. Virol.*, doi:10.1128/JVI.00384-09). Also, the VRC's DNA vaccine candidate consists of six different plasmids, one for each encoded antigen (Gag, Pol, Nef, and Env from clades A, B, and C), and dividing the vaccine in this way has been shown to reduce the bias toward Env-specific CD4⁺ T-cell responses by enhancing Gag-specific and Nef-specific CD4⁺ T-cell responses (*Vaccine* 25, 4085, 2007).

Other macaque studies using the VRC DNA/Ad5 candidates have also produced different results than those obtained by Barouch. In the most cited study, the DNA/Ad5 prime-boost was shown to perform comparably to Ad5 alone (*Sci-*

ence 312, 1530, 2006). A poster by Diane Bolton from the ImmunoTechnology Section at the VRC also included a comparison of the VRC's DNA/Ad5 to just Ad5, albeit on a slightly different schedule (two Ad5 booster shots were given). Again, while DNA/Ad5 was not shown to be superior to Ad5 alone in this study, the two regimens performed equally well and reduced viral load set points by one to two logs compared to placebo. The US Food and Drug Administration is currently reviewing the HVTN 505 protocol.

Building a better antigen

Barouch also presented results from a mosaic vaccine study in rhesus macaques designed to achieve optimal coverage of various 9mers of a given protein. In theory, he said, a mosaic vaccine with sequences encoding a given number of mosaic antigens will have better breadth and coverage than a vaccine candidate encoding the same number of consensus antigen sequences or one encoding the same number of naturally occurring antigen sequences (*Nat. Med.* 13, 100, 2007).

In this study, Barouch used an Ad26 vector with sequences encoding two mosaic antigens for each of the three HIV proteins Gag, Pol, and Env, designed to optimize coverage of the global HIV-1 M group, which represents the vast majority of all global HIV sequences. Macaques were immunized intramuscularly with the mosaic vaccine candidate; an Ad26 vector with one set of consensus antigen sequences for Gag, Pol, and Env; or an Ad26 vector with one set of natural sequences for these proteins that had the broadest 9mer coverage of all clade CHIV sequences in the Los Alamos National Laboratory HIV sequence database.

Barouch found that the mosaic vaccine generated CD8⁺ and CD4⁺ T-cell responses directed at about three to four times more epitopes than the other two vaccines, using a pool of global potential T cell epitope peptides developed by the HIV Vaccine Trials Network that represents 85% of the global viral sequences. The mosaic vaccine increased the breadth of the immune responses as well as their depth, which is the simultaneous induction of responses to different versions of the same epitopes. The mosaic vaccine also showed a broader response than the other two vaccines to consensus Gag proteins from clades A, B, and C, and to two natural clade C Gag proteins.

These results suggest that “the evaluation of rare serotype Ad vectors expressing HIV mosaic

antigens optimized for global coverage may be warranted,” said Barouch.

STEP by step

Susan Buchbinder, principal investigator of the STEP trial, provided another update on this now notorious Phase IIb trial of Merck’s Ad5-based vaccine candidate, MRKAd5. Receipt of MRKAd5 was associated with enhanced susceptibility to HIV infection, most significantly among uncircumcised MSM, who had pre-existing immunity to the Ad5 vector. An explanation for these findings has so far been elusive.

Some scientists have highlighted the finding that placebo recipients in the STEP trial with the highest pre-existing Ad5 antibody titers had the lowest risk of HIV acquisition, and perhaps some unknown factor renders individuals with high anti-Ad5 titers less susceptible to HIV infection. But based on several analyses, Buchbinder said there was no association between baseline Ad5 antibody titers and risk of HIV acquisition. Herpes simplex virus (HSV)-2 seropositivity was associated with a roughly two-fold increased risk of HIV infection among STEP participants, but it did not explain the enhanced risk of infection among vaccine recipients either.

Buchbinder then unveiled new data from the extended follow-up of STEP participants from October 2007 to January 2009, the period after the volunteers were unblinded. Buchbinder said that while there was a slight drop in high-risk sexual activity after unblinding, risk behavior quickly returned to the level previously observed during the trial. The rate of HIV acquisition among MSM remained high, with 48 new infections occurring during this period—26 among MRKAd5 recipients and 22 in the placebo group. Buchbinder plotted the occurrence of these infections over time, and showed that the difference in HIV incidence between vaccine and placebo groups seems to be disappearing. But she emphasized that while this finding may offer some reassurance that the enhancement effect of Ad5 is time-limited, the results must be interpreted cautiously because of the small numbers of volunteers in these groups. Buchbinder also reported that 12 additional infections have occurred among women volunteers (only one had occurred when the first trial results were announced) and these were divided evenly between the vaccine and placebo groups.

In a complementary presentation, Julianna McElrath from the Fred Hutchinson Cancer

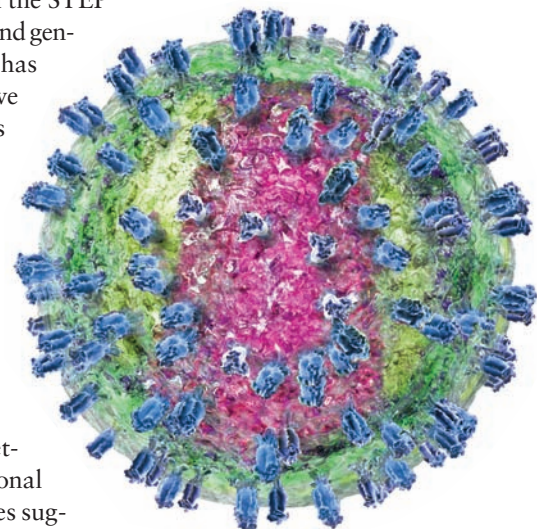
Research Center discussed ongoing immunological studies. Although no immune parameters have been identified that discriminate between individuals that became infected versus those that did not, some hints of a vaccine effect on viral load have emerged. These findings derive from a very small number of STEP participants with favorable HLA alleles (HLA B*57 and HLA B*27). In this small subset of individuals, it appears that receipt of MRKAd5 led to slightly lower set-point viral loads compared to placebo recipients with the same HLA alleles. A collaboration between Bruce Walker, director of the Ragon Institute, and David Heckerman at Microsoft Research has also suggested that the targeting of certain epitopes by vaccine recipients led to better virus control (see *Cannassing CROI, IAVI Report*, Jan.-Feb. 2009).

McElrath also presented data generated with Mark Connors, chief of the HIV-specific immunity section at NIAID, who developed a new assay that measures the ability of CD8⁺ T cells to kill HIV-infected CD4⁺ T cells *in vitro* (see *Research Briefs, IAVI Report*, Jan.-Feb. 2009). When this assay was used to test the efficacy of CD8⁺ T-cell responses induced by two doses of MRKAd5 in HVTN 071 (sufficient cells were not available from the STEP trial), the extent of cell killing was poor and generally comparable with what Connors has reported for individuals with progressive HIV infection. McElrath noted that it’s likely that vaccine candidates will instead need to induce the type of efficient killing Connors has documented among elite controllers.

Mucosal protection

Chris Miller, a professor at the School of Veterinary Medicine at the University of California in Davis, and Barbara Felber, chief of the human retrovirus pathogenesis section at the National Cancer Institute, both presented studies suggesting that, in Felber’s words, the dogma that only mucosal vaccination can induce mucosal immune responses, may not be entirely true.

Miller presented studies exploring the possible mechanism for how IV vaccination of macaques with an SIV/HIV hybrid strain known as SHIV 89.6 protects about 60% of macaques from SIV-mac239 vaginal challenge (*J. Virol.* 82, 11181, 2008; *Mucosal Immunol.* 1, 219, 2008). Previously he had found that for six months after SIV challenge, the viral load of 60% of SHIV 89.6 vac-

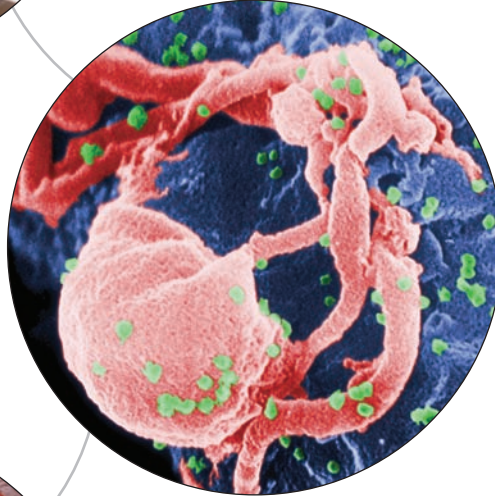


Continued on page 14



A Living

History



“
We didn't know it was a virus, we didn't have a virus, but it was acting like a virus, and it was destroying the immune system.
”

“
I don't see myself as a politician, I see myself as an honest broker of science. That's the reason I think I've been able to be effective.
”



of AIDS

Vaccine



“
Research is fundamentally a bunch of failures with an occasional bright light of a success.
”



Research

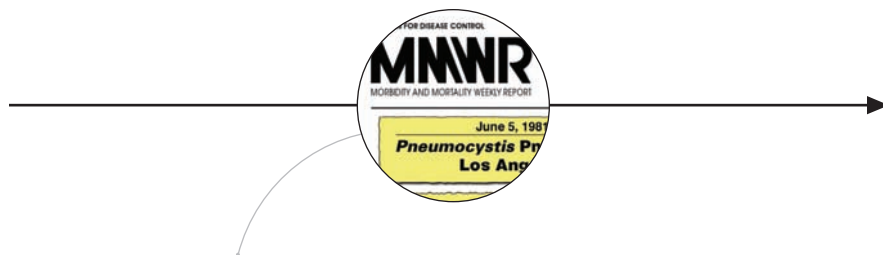
Anthony Fauci has been at the forefront of AIDS vaccine research for decades. When AIDS surfaced in 1981, he, like many other

scientists and physicians, was drawn to the mysterious illness, which has now claimed more than 25 million lives—more than the populations of Ghana or Taiwan. Since 1984, he has served as director of the National Institute of Allergy and Infectious Diseases (NIAID) at the US National Institutes of Health (NIH). He has been a key advisor to US presidents on global AIDS issues and was a leading architect of the US President’s Emergency Plan for AIDS Relief (PEPFAR). Born on Christmas Eve, 1940, and raised above a pharmacy in Brooklyn, New York, he earned a medical degree from Cornell Medical College. Fauci, a marathoner, avid fisherman, and father of three, has invited AIDS activists into his home. Last year, he presided over a US\$4.4 billion budget—roughly a third of it dedicated to HIV/AIDS research.

He ranks among the top 10 most cited HIV/AIDS researchers in the world and has received numerous awards, including the Presidential Medal of Honor for leading the fight against HIV/AIDS and the Lasker Award for Public Service. “I don’t see myself as a politician, I see myself as an honest broker of science. That’s the reason why I think I’ve been able to be effective,” Fauci said during a January 2009 interview with *IAVI Report* Managing Editor Kristen Jill Kresge and Science Writer Regina McEnery, which served as the basis for this first installment in the Living History series and features Fauci in his own words.

Additional chapters, each featuring a recounting of historic milestones in the search for a vaccine by some of the most prominent players in the field, will appear in upcoming issues.

➤ A video podcast with Fauci can be viewed or downloaded at www.iavireport.org



June-July 1981

In a chilling prologue to one of the worst pandemics in human history, the Morbidity and Mortality Weekly Report issues a brief report about an unusual spate of pneumocystis carinii pneumonia (PCP) infections among “five gay, otherwise healthy men” from Los Angeles. Fauci, a young immunologist at NIAID, is instantly curious because PCP has almost exclusively been seen in severely immunocompromised individuals, and these men have no known medical history that could have predicted this unusual diagnosis. A month later, 46 more cases are reported, in Los Angeles, San Francisco, and New York. Those affected are now also developing Kaposi’s sarcoma, a cancer caused by a herpes virus, which becomes a hallmark of this new disease.

Fauci: For the first time in my medical career I actually got goose pimples. As more cases were being reported, I decided in the summer of 1981 that I would change the direction of my laboratory and focus only on this unusual disease called at that time Gay Related Immunodeficiency Disease, or GRID. We didn’t know it was a virus, we didn’t have a virus, but it was acting like a virus, and it was destroying the immune system.

My mentor, Dr. Sheldon Wolff, who recruited me to the NIH, called me and said, ‘You’re crazy. You have such a great career in front of you. Do me a favor, don’t give up your day job.’ Well, I did give up my day job, and I essentially went full time studying HIV in the lab until 1984 when I became director of NIAID.

Images in this article reprinted with permission from the National Institute of Allergy and Infectious Diseases, the Morbidity and Mortality Weekly Report, the US Centers for Disease Control and Prevention, IAVI, and Science magazine.



April 23, 1984

Reported cases of the mysterious new disease, now called AIDS, top 4,000 in the United States and reported deaths surpass 1,800. Hemophiliacs, infants born to HIV-infected mothers, and injection-drug users, in addition to men who have sex with men (MSM), are at high risk, and observations of this same disease from sub-Saharan Africa hint at the developing tsunami that would soon overwhelm swaths of the continent. Then-US President Ronald Reagan is criticized for ignoring the burgeoning epidemic. But the public health community is encouraged when scientists in both the US and France announce separately that they have discovered a new retrovirus as the cause of AIDS. At a press conference highlighting the achievements of the US team led by Robert Gallo from the National Cancer Institute, Reagan's Health and Human Services Secretary Margaret Heckler tells the media that a vaccine candidate will be ready for testing within two years.

Fauci: Even though it was interpreted that she said we would have a vaccine in two years, she really said we would have a vaccine *ready for testing* in two years. Subsequently, when all of the issues began to emerge about how difficult it would be to get an AIDS vaccine, she suffered slightly justifiably, but mostly unjustifiably, as having predicted that we would actually have a vaccine ready for use and distribution. Two to three years after that announcement, [a trial testing] one of the early, unsuccessful HIV envelope candidates actually started right here at the NIH. It certainly wasn't the right vaccine, but it's just interesting that many years later when you talk about the big gaffe that she made, in reality it wasn't.

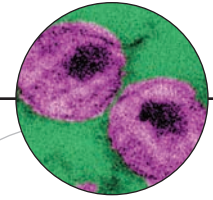


Spring 1985

With no drugs or viable vaccine candidates available to treat or prevent the growing epidemic, Fauci approaches then-NIH Director James Wyngaarden about quadrupling funds to jump-start efforts to combat the elusive virus. To handle the escalating research efforts, NIAID later creates the Division of AIDS within NIAID. These moves are criticized by some scientists, but Fauci's decision is later justified as the epidemic evolves into one of the worst in history.

Fauci: We got an extra \$60-\$100 million, which at the time was an enormous amount of money. A lot of people got angry that the new director of NIAID was putting all of this money into AIDS. Now we're spending, appropriately, \$2.9 billion a year at the NIH on AIDS. But at that time people thought this was just a curiosity of a disease and that it would not have a major public health impact, and of course history has shown that that is absolutely not the case.

When you're living through history you often don't realize that what you are experiencing is an historic event. I think if you read the history books and you see people who are involved in things that ultimately turn out to be historic, rarely did they realize that what they were doing was something historic. We are living through one of less than a handful of the most devastating pandemics ever to confront human civilization—pandemic flu of 1918, smallpox, the plague, and HIV. Every year that goes by 2.7 million people get infected with HIV. So there's a lot of passion in wanting to do something about it.



June 17, 1994

HIV's virtually unrivaled ability to mutate makes traditional vaccine strategies, such as the use of live attenuated or killed versions of the virus, both risky and impractical. Instead US biotechnology company Genentech develops an AIDS vaccine candidate comprised of HIV gp120 and approaches NIAID about funding a Phase III trial—the first ever efficacy trial of any AIDS vaccine candidate. But based on the data, Fauci refuses to fund the study.

Fauci: When we were considering this Phase III trial, understandably, there was a lot of play on emotion. How can we sit here and do nothing? That's a very strong reason to push on the empiric approach, and I wasn't against that, but I was starting to realize that the scientific data was really weak.

In general, classical vaccinology is based on the premise that we see what the body does in natural infection and we try to mimic it. We were focusing on the classic paradigm, which is understandable, because that's how vaccines have been developed for decades. But, as we were developing AIDS vaccines, we started to see that some of those classic paradigms didn't hold. It was very difficult in the natural state to develop neutralizing antibodies. Essentially nobody eradicates the virus from their body. There's a small percentage of long-term nonprogressors who seem to control virus replication, but inevitably the disease progresses and the immune response is inadequate. We still don't know why the immune system is incapable of mounting a response that with any other virus would ultimately be protective.



June 9, 1999

NIAID establishes the Vaccine Research Center (VRC) at the NIH to focus primarily on development of an AIDS vaccine. The VRC is the result of a 1997 pledge from then-President Clinton to develop an AIDS vaccine within 10 years.

Fauci: Harold Varmus and I, and a few others, went down to the White House and were briefing Vice President Al Gore and President Bill Clinton about HIV/AIDS. I was actually showing him a now-famous picture of me explaining what CCR5 is and how the virus binds to CD4 and then changes its conformation and goes to CCR5. I told him this has really important relevance for the development of a vaccine because it's those cryptic and then exposed epitopes that we can't seem to make a good immune response against. And as we were walking out to the Rose Garden, the president said, 'So what is it that you really need?' I said, we need to accelerate our effort on vaccine development and the best way we can do that is to have an entity where we can go from fundamental basic research right up to the early phases of testing. If we can get a critical mass of the best people in one place physically, first here on campus and then perhaps even in the extramural community, that would be a big contribution. So they said, 'Do it.' It was the fastest time from somebody promising us a building to actually getting it.

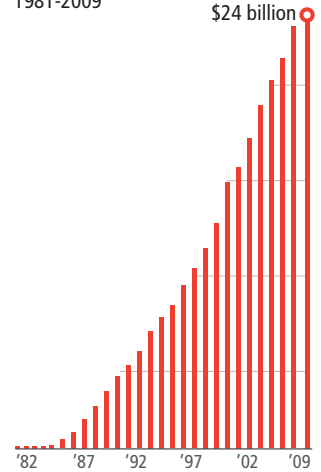


October 1, 2002

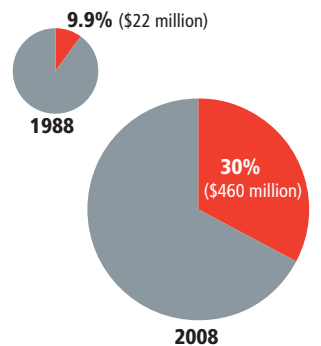
NIAID assumes control of the US Department of Defense's HIV Research and Development Program, which had been preparing for a Phase III efficacy trial in Thailand to test Sanofi Pasteur's canarypox-based vaccine candidate in a prime-boost combination with a gp120 candidate developed by VaxGen. Many researchers publicly criticize NIAID's eventual decision to move ahead with this trial since there was little evidence that this prime-boost strategy would be effective.

Fauci: I think the scientific data [with this prime-boost strategy] was a little bit stronger [than for just gp120]. In a perfect world, if there were not commitments that had been made to other nations and to other agencies, the decision may have been different. When that decision was made, we were learning more about how problematic this virus is. At the same time there was a push, driven by the historic success of empiric approaches and the compelling need in certain countries for a vaccine. It's critical to understand that. You have a country that says, 'You people promised you would help us with this vaccine. We know the chances might be slight, but slight is better than nothing.' There were a lot of people who were saying in a very objective way—I felt somewhat that way myself—that this has a really small chance to be successful. But you've got to balance that against other issues. Would I have done a trial like that in the US? No way, because the infection rate in the United States is significantly lower than what it was at the time in Thailand.

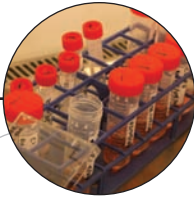
Federal Funding for HIV/AIDS
1981-2009



NIAID AIDS Vaccine Funding
As a percentage of NIAID AIDS funding



Source: NIAID



July 14, 2005

NIAID announces \$300 million in funding over seven years to establish a virtual consortium of research laboratories known as the Center for HIV/AIDS Vaccine Immunology (CHAVI). The Center was based on recommendations by the Global HIV Vaccine Enterprise that Fauci and 23 other AIDS researchers proposed two years earlier to better coordinate research and promote big science efforts to overcome key immunological roadblocks to vaccine development.

Fauci: Even at the time that we were doing empiric clinical trials, the science was evolving and we were realizing that there were so many things that we needed to discover. So we came up with some recommendations, which were ultimately published in a now very well-quoted article in *Science*. One of the things that we recommended was to have centers modeled in an extramural and collaborative way, like what we had done with the VRC, and that one of the centers would be involved in immunology since it's such an important component. When the center came about, a number of people said we were putting in too much money, which is very interesting because many of those people were in on the recommendation that we should have this kind of center. I think, at the end of the day, most people feel that CHAVI is very productive.

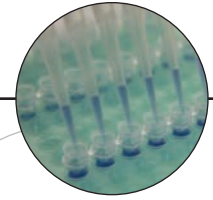
Cohort	Vaccin Infection
First 1,500 person cohort, anti-Ad5 antibody levels >200	28
and 1,500 cohort.	21

September-November 2007

A large Phase IIb proof-of-concept trial of 3,000 individuals known as STEP shows Merck's adenovirus serotype 5 (Ad5) vector-based vaccine candidate (MRKAd5) is not effective. Subsequent findings, released two months later, suggest the vaccine might have led to an increased susceptibility to HIV among uncircumcised men with pre-existing immunity to the Ad5 vector. The results of the trial are a major disappointment to many and highlight some of the major gaps in AIDS vaccine strategies. Fauci first learns the results of the STEP trial when he receives a phone call from Larry Corey, principal investigator of the HIV Vaccine Trials Network.

Fauci: Larry sounded like he had been hit by six trucks. I've never heard him sound so bad. He said, 'Tony, you're not going to believe what I'm going to tell you. There's nothing there. Not even a hint or a whiff of any effect. And brace yourself, it looks like there may even be an increased risk among some of the people—particularly those with high adenovirus titers.' Although some of us, myself included, were really less than cautiously optimistic, we were hoping that we would see some signal that would allow us to build on the next generation of a similar type of vaccine. We didn't expect that the first look at the data would show essentially abject lack of success, as well as a spectre of risk.

My job was to remind people that research is fundamentally a bunch of failures with an occasional bright light of a success and to tell them that we're not going to give up on vaccines.



March 25, 2008

The STEP trial, which was funded in part by NIAID, sets the field on a new course and sparks debate about the prospects of T-cell based vaccine candidates. NIAID announces plans to shift funding from product development to basic discovery at a daylong Summit on HIV Vaccine Research and Development.

Fauci: There were some people who were inappropriately saying we might as well not do any vaccine research. That's the absolute wrong response. Not only are we not going to stop HIV vaccine research, we're actually going to accelerate it and put more money into it, however, we're going to take a look at what we're doing. So we brought in a group of people who had been laboring at this for some time, as well as some people with new ideas. Since natural infection hasn't proved the concept we've got to do better than natural infection. The days of the empiric, give me a product and I'll test it in a big trial, essentially are over. That doesn't mean that clinical trials are over because clinical research and clinical trials can be part of discovery. Small trials that look at immune responses, the nature of the response, and its breadth and depth, those things are part of discovery.



July 17, 2008

In the immediate aftermath of the STEP results, several planned trials are postponed. One of these, known as PAVE 100, was a Phase IIb test-of-concept trial of a DNA/Ad5 prime-boost regimen developed at the VRC. Following the STEP trial results, the PAVE 100 trial protocol was altered to include only circumcised MSM in the US with no pre-existing Ad5 antibodies, but Fauci decides the data is insufficient to support a trial of this size and scope. A protocol for an even smaller trial is still under consideration.

Fauci: PAVE was different in several ways from STEP. First of all, it [the Ad5-based candidate] had envelope in it. Secondly, it's a DNA prime followed by an adenovirus boost. The animal model showed clearly that it had an effect in both simian immunodeficiency virus (SIV) and SHIV (an SIV/HIV hybrid). It wasn't an overwhelming, knock-me-off-my-chair effect, but it clearly was quantitatively and qualitatively a bit better than the candidate tested in the STEP trial.

In looking at the data, I believed that there was enough difference to warrant a truncated, lean but mean, proof-of-concept trial. The first time, they came back with a trial that in my mind was still too large because it was powered to determine the correlates of immunity. I said, show me a trial that is powered to show if the candidates either work or don't work. If the candidate works, then we'll build on that trial.



April 2009

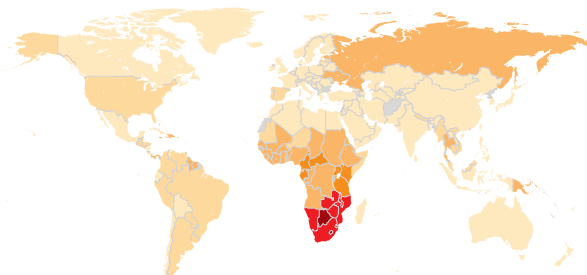
Nearly 28 years after the first five cases of AIDS are reported, the relentless search for a vaccine continues. Fauci is now serving his fifth president. His goal remains the same, even though he acknowledges that an AIDS vaccine may not, in fact, be possible.

Fauci: Don't be frightened but we may not ever have an AIDS vaccine in the classical sense of being 95% protective. Am I diminishing our efforts? No. In fact, I'm accelerating the vaccine research efforts, at least on the part of NIAID. Unlike other vaccine endeavors, we're still in the stage of discovery, and discovery is haphazard—sometimes blind alleys, sometimes Eureka moments—and completely unpredictable. We still don't know how, why, or if a body makes a robust neutralizing antibody and T-cell response that can both block acquisition and prevent disease progression. The reason we don't know this, is because the body doesn't do it in natural infection. With other viruses, nature tells us just follow me and I'll lead you to a vaccine. With HIV, nature is telling us if you follow me, you're going to be in trouble. We're going to have to push the envelope with HIV vaccinology in ways that we never had to do before. I feel that as we probe the scientific secrets of HIV, we may get there. If we can, with our own capabilities, intellect, and drive, manipulate the immune system to do something that natural infection doesn't seem to be able to do, what else can we do with the immune system? The vista is almost infinite.

 **A video podcast with Fauci can be viewed or downloaded at www.iavireport.org**

HIV Prevalence Worldwide (Adults, 2007)

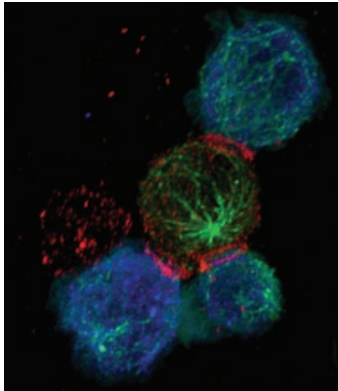
Lowest  Highest



Adults and Children Living with HIV (2007 estimates)

North America	1,200,000
Caribbean	230,000
South America	1,700,000
Europe	730,000
North Africa and Mideast	380,000
Sub-Saharan Africa	22,000,000
Northern Asia	1,500,000
Southern Asia	4,200,000
East Asia	740,000
Australia & Oceania	74,000
TOTAL	33,000,000

Source: The Joint United Nations Programme on HIV/AIDS (UNAIDS)



▲ **POLYSYNAPSES FORMED BETWEEN AN HIV-INFECTED T LYMPHOCYTE AND THREE TARGET CELLS** HIV Gag: red; tubulin: green; target cells: blue. Image by Dominika Rudnicka, Nathalie Sol-Foulon, and Olivier Schwartz, at the Institut Pasteur

HIV can be transmitted as cell-free or cell-bound virus, and researchers are now starting to literally picture how cell-bound virus is transmitted between cells through virological synapses (see *Research Briefs*, this issue). At Keystone, Olivier Schwartz, head of the virus and immunity unit at the Institut Pasteur in Paris, France, showed that in cultured human cells, the most common mode of cell-bound HIV transfer is between an infected cell contacting one or several (polysynapses), target cells (*J. Virol.* doi:10.1128/JVI.00282-09).

In polysynapses, Schwartz found that viral Gag particles move simultaneously from the infected cell to several target cells. Gag and Env were colocalized at the contact zone between the cells, suggesting that infectious particles are there. Using transmission electron microscopy, he observed viral particles, at various stages of budding and maturation, accumulate in the extracellular cleft between an infected cell and target cells of a polynapse. Three-dimensional reconstructions of fluorescent images of Gag proteins at virological synapses often showed them arranged in a ring-like structure at the contact zone (see above). —AvB

Continued from page 7

inated macaques remained below 10,000 copies/ml, two to three logs lower than unvaccinated controls (*J. Virol.* 77, 3099, 2003). These animals were protected from progression to AIDS, and did not show progressive CD4⁺ T cell loss compared with either vaccinees with viral loads higher than 10,000 copies/ml, or controls.

The current studies found no definitive correlates of protection in blood, but in the vagina Miller found that before challenge, 60% of the vaccinated monkeys had SIV Gag-specific CD8⁺ T-cell responses, and all of them had SIV Gag-specific CD4⁺ T-cell responses. “The fact that the CD4s were [in] 100% [of the monkeys] and we are only getting protection in 60% suggests that the CD4s aren’t the protective cell type but the CD8s are,” Miller said.

Depletion of CD8⁺ T cells at the time of challenge resulted in massive virus replication, including enhanced virus levels in the vagina and the cervix compared with unvaccinated controls. This, along with the observation that all vaccinated animals had CD4⁺ T-cell responses in the vagina but only 60% were protected, suggests to Miller that CD4⁺ T-cell responses might enhance viral replication in the genital tract. “There is a fine balance between protection and enhanced viral replication” elicited by the vaccine, he said.

These experiments also show that one doesn’t necessarily have to vaccinate via a mucosal route to elicit mucosal immune responses. “We are IV inoculating [these animals] with a replicating virus, and that’s inducing these mucosal T-cell responses,” Miller said. “The more we look at this stuff the more we find that any time we elicit a strong systemic immune response, a lot of it spills off into the mucosa.”

Felber also found that systemic vaccination can lead to mucosal immune responses. She observed that intramuscular electroporation of a DNA vaccine encoding the SIVmac239 proteins Gag, Pol, Env, and Nef-Tat-Vif, induced cellular and humoral immune responses in the bronchoalveolar lavage (BAL) of rhesus macaques as well as cellular immune responses in the rectal mucosa. The cellular immune responses in the BAL were between 2 and 40 times higher than in blood, and were still observed 45 weeks after vaccination, much longer than the two weeks that immune responses are detectable after classical intramuscular injection. In the BAL, the vaccination also induced Gag-specific IgA antibodies.

She said this is the first time that electroporation has been shown to induce mucosal immune

responses. “There has been this dogma that in order to get mucosal immune responses, you need to deliver your antigen via dendritic cells or mucosal tissue,” said Felber. “[But with] a very efficient delivery like intramuscular electroporation, we find beautiful mucosal cell and humoral immune responses.”

Antibodies: Better together or alone?

Johannes Scheid from Rockefeller University presented results from a recently published study that suggests that antibodies in HIV-infected people can work in concert to fight the virus (*Nature* 458, 636, 2009). Scheid and colleagues isolated gp140 trimer-binding IgG memory B cells from six HIV-infected individuals with high titers of broadly neutralizing sera against different HIV strains. The researchers found between 22 and 50 independent memory B cell clones in each person, and made monoclonal antibodies from them. These B cell clones did not include any of the four already known broadly neutralizing antibodies, all of which also bind the gp140 trimer.

By themselves, the isolated antibodies had some neutralizing activity, although only rarely against HIV strains that are more difficult to neutralize, Scheid said. They were also much less potent than the four known broadly neutralizing antibodies, he added. Overall, the neutralizing activity of the isolated antibodies was less broad than what was observed for the original sera. In some cases, recombining equal amounts of some of the monoclonal antibodies at high concentrations reconstituted the broad neutralizing activity found in the original serum from the same individual, suggesting that antibodies can work in concert to achieve broad neutralization. Still, Scheid said, the findings do not necessarily mean that the search for one “golden” broadly neutralizing antibody should be abandoned.

And indeed, that search is still on. Dennis Burton, a professor of immunology and molecular biology at the Scripps Research Institute, presented results of an effort by IAVI’s AIDS Vaccine Design and Development Laboratory in Brooklyn, New York, and its Neutralizing Antibody Center, in La Jolla, California, which he heads, that led to the identification of two new broadly neutralizing antibodies. As part of its research study, known as protocol G, which seeks to identify new broadly neutralizing antibodies, IAVI screened sera collected from individuals who have been HIV infected for at least three years against panels of viruses. In collaboration with the company Spaltudaq, now

called Theraclone Sciences, researchers then isolated IgG memory B cells from the most promising serum samples, made monoclonal antibodies from them, and screened the monoclonal antibodies for neutralization and binding to gp120 and gp41 Env proteins. In the study, antibodies that neutralized certain viruses often did not bind the gp120 protein of the same virus, suggesting that binding doesn't necessarily preclude neutralization. The fact that the initial screen was for neutralization and not for gp120 binding might have contributed to the project's success, Burton believes.

Two antibodies did particularly well, even compared with the four already identified broadly neutralizing antibodies. "There is great interest in these antibodies," Burton said. "They don't hit everything by any means, but they do hit a lot and what they hit is often very potent." Burton said the two newly identified antibodies bind to a new epitope on the Env trimer, where there are perhaps fewer problems with accessibility than with some of the known broadly neutralizing antibodies that bind closer to the membrane, or are more sterically obstructed.

More work on antibodies was presented by Peter Kwong, chief of the structural biology section at the VRC. Kwong is trying to understand why antibodies that bind to the CD4 binding site on HIV gp120 do not necessarily neutralize the virus. He found that one such antibody called b13 binds the gp120 monomer at an angle about 15 degrees different from that of the broadly neutralizing antibody b12. This difference, though subtle, induces a conformational change in the gp120 monomer. This b13-induced conformation of the gp120 monomer is not easily compatible with the Env trimer, which means that it likely cannot be induced—or exist—in the context of the trimer, said Kwong. This explains why b13 doesn't neutralize the virus, even though it binds the gp120 monomer.

So to be able to neutralize, he said, antibodies have to precisely recognize the vulnerable initial site of CD4 attachment on the Env trimer. "You have to be right on, and we have figured out the mechanism of why you have to be right on," Kwong said, adding that to develop appropriate immunogens to elicit antibodies that effectively target this initial site of CD4 attachment, it's important to understand what works, but also what doesn't work. "If you only watch Tiger Woods, you have no idea how difficult it is to hit that ball just right," he joked.

The studies presented by Scheid and Burton suggest to Kwong that there are two possibilities

of how broad neutralization could be achieved: Either there are few broadly neutralizing antibodies that neutralize everything, or many antibodies with weak activity that work together. "Both possibilities might be true," he said.

HIV-specific T cells in the gut

Barbara Shacklett, associate professor at the University of California in Davis, presented data on HIV-specific T-cell responses in the gut of HIV-infected individuals. Shacklett's laboratory has developed expertise in the careful segregation of CD4⁺ and CD8⁺ T cells from gut tissue, which allows these populations to be analyzed in detail. In a study led by postdoctoral researcher April Ferre, Shacklett's group used the approach to compare responses between individuals who control HIV replication in the absence of treatment and those with progressive infection, with or without treatment.

The study included 17 elite controllers (viral loads less than 75 copies/ml), 11 viremic controllers (viral loads between 75 and 2,000 copies/ml), 14 non-controllers (viral loads >10,000 copies/ml), and 10 individuals with undetectable viral loads on antiretroviral therapy (ART). Gag-specific CD8⁺ T-cell responses were evaluated in rectal mucosa and blood and Shacklett reported that, while there was no difference in blood, mucosal Gag-specific CD8⁺ T-cell responses were significantly higher in the controllers, both elite and viremic, than in non-controllers and individuals on ART. Similarly, Gag-specific CD8⁺ T cells expressing multiple cytokines/chemokines were significantly higher in the rectal mucosa of controllers.

A novel finding from this study was that the CD8⁺ T-cell responses among controllers were associated with particular class II HLA alleles, DRB1*13 and/or DQB1*06, which present antigens to CD4⁺ T cells. One or both of these alleles was present in 70% of elite controllers, 45% of viremic controllers, and 8% of non-controllers. In a related poster, Ferre showed that these alleles were also associated with the presence of stronger Gag-specific CD4⁺ T-cell responses in the gut of controllers. Shacklett's group is now further exploring the role of class II HLA alleles among HIV controllers.

Non-pathogenic SIV infection

Several presentations focused on research into the mysteries of non-pathogenic SIV infec-

If you only watch
Tiger Woods,
you have no idea
how difficult
it is to hit that
ball just right.

— Peter Kwong

[HSV-2 INFECTION AND HIV RISK]

The finding that infection with herpes simplex virus (HSV)-2 raises the risk of acquiring HIV by about two- to three-fold, dependent on sex and route of exposure (*AIDS* 20, 73, 2006), led directly to large randomized trials to evaluate the impact of HSV-2 suppression with acyclovir on HIV infection. The hope was that acyclovir, by suppressing HSV-2 reactivation, would decrease the incidence of HIV infection, but this strategy proved ineffective (see *Clues from CROI, IAVI Report, Jan.-Feb. 2008*).

Larry Corey, principal investigator of the HVTN, set out to try to understand why by conducting a study with 15 HSV-2 infected individuals, nine untreated and six receiving chronic suppressive therapy with acyclovir. He found that even in the presence of acyclovir, HSV-2 infection was associated with “very large nests of CD4⁺ T cells” under the dermis in areas of prior lesions—two- to 32-fold more CD4⁺ T cells compared to controls. The proportion of CCR5-expressing CD4⁺ T cells was also higher at these sites in 14 of the 15 study participants. These CD4⁺ T cells were largely HSV-2-specific and appeared to be engaged in active and effective immune surveillance, according to Corey.

He also reported that DC-SIGN-expressing dendritic cells were enriched in these samples, clustered with the CD4⁺ T cells. This indicates that while acyclovir can clearly prevent the incidence of symptomatic HSV-2 reactivation, it does not abrogate the need for local immune control of HSV-2. As a consequence, HSV-2 infection causes an increased mucosal presence of CCR5-expressing CD4⁺ T cells, which are optimal targets for HIV infection. Corey concluded that the ideal approach to reducing the affect of HSV-2 on HIV acquisition would be to prevent HSV-2 infection altogether. —RJ

tion. Guido Silvestri, associate professor of pathology, microbiology, and immunology at the University of Pennsylvania, discussed sooty mangabeys, natural hosts of SIV viruses that are pathogenic in macaques and direct antecedents of HIV-2 in humans. Silvestri explained that the lack of disease progression in mangabeys is not due to control of viral replication—viral loads in these animals are as high or higher than those associated with disease progression in HIV-infected humans or SIV-infected macaques. Rather, what distinguishes non-pathogenic infection is the absence of persistent immune activation. Silvestri spoke about one hypothesis to explain this phenomenon—lack of IFN- γ production by dendritic cells, which is essentially a lack of an innate immune response to SIV.

To evaluate the merits of this, Silvestri designed a study to look at whether acute SIV infection of sooty mangabeys is associated with an absence of immune activation or if activation occurs but is then actively downmodulated. He analyzed gene expression in sooty mangabeys and macaques challenged with either SIVsm or SIVmac239.

Silvestri reported that acute infection of sooty mangabeys was associated with “massive changes” in the transcriptional profile of multiple genes, which were similar to those seen in the macaque groups. The gene expression profiles, however, diverged in chronic infection. Silvestri showed that IFN-stimulated genes were upregulated to the same degree or even higher in sooty mangabeys than in macaques during acute infection, but returned to baseline during chronic infection only in the mangabeys. Silvestri concluded that the data support a model in which there is active downmodulation of immune activation in sooty mangabeys during the transition from acute to chronic infection. Contrary to the idea that the immune system simply ignores the virus, Silvestri stressed that “the host is responding in a very vigorous way to infection.”

Silvestri also presented data indicating that CD4⁺ T cells express less CCR5, the key co-receptor for virus entry, in sooty mangabeys. This is not the case for CD8⁺ T cells, where CCR5 expression levels mirror humans. When mangabey CD4⁺ T cells are activated, CCR5 upregulation is also delayed compared to humans and other monkey species.

Battlefield maps

Ashley Haase, head of the Department of Microbiology at the University of Minnesota, provided an update on his studies of SIV pathogenesis. Of particular interest for vaccine research, Haase has been exploring the facets of an effective SIV-specific CD8⁺ T-cell response using detailed analyses of tissue samples. Here he introduced a visually compelling innovation he describes as a “battlefield map,” which involves overlaying images of tissue sections stained for viral RNA to identify SIV-infected cells, then stained for SIV-specific CD8⁺ T cells using a modified version of the tetramer assay. Haase has dubbed the method ISTH, to indicate the combination of *in situ* tetramer staining to identify the CD8⁺ T cells and *in situ* hybridization to locate and quantify virus-infected cells.

Haase shared battlefield maps from a study of female macaques challenged intravaginally with SIVmac239. The decline in SIV viral load from peak levels at around days 10-14, to day 21, was correlated with the detection of conjugates of SIV-specific CD8⁺ T cells and SIV-infected cells.

In addition to the images, Haase generated quantitative data by calculating effector-to-target (E:T) ratios. Using this technique, he found that the E:T ratio was correlated with the SIV viral load decline in acute infection. In all but one animal, the highest E:T ratios were attained in the cervical tissues, where initial exposure had taken place.

Haase also outlined results from a collaboration with immunologist Rafi Ahmed at Emory University using the murine lymphocytic choriomeningitis virus (LCMV) model. Their study involved two LCMV variants, the Armstrong strain and clone 13; the former only causes an acute infection, which the immune response rapidly clears, while the latter establishes a chronic infection. Haase used the ISTH technique to demonstrate that these differences are driven by the expanded target cell range of clone 13, which infects many more cells than the Armstrong strain. As a result, the E:T ratio is insufficient to contain clone 13. With the Armstrong strain, the number of effector cells quickly exceeds the number of infected cells and the virus is cleared.

Haase said the ultimate goal of this work is to gain an understanding of how many effector T cells need to be induced by an HIV vaccine to mediate viral clearance or control; in Haase’s words, to achieve “enough, and soon enough” (*Science* 323, 1726, 2009). ■

Vaccine BRIEFS

CAVD Reports Progress

THE COLLABORATION FOR AIDS VACCINE DISCOVERY (CAVD), an international research network created in 2006 by the Bill & Melinda Gates Foundation to accelerate development of an AIDS vaccine, has recently issued the first-ever cumulative review of its progress. The CAVD now comprises 400 investigators in 21 countries, with total funding exceeding US\$327 million, representing the majority of the Foundation's support for AIDS vaccine research and development. When it was created, the CAVD model included 16 funded institutions but it has since grown to include 19 primary grantees that all work with a number of other collaborating institutions around the world.

The report, available at www.cavd.org, provides an overview of the scientific and operational (legal and business) progress made by the network of nearly 100 public and private research institutions involved in the CAVD over the past two and a half years. The researchers involved in the CAVD are exploring a range of approaches to AIDS vaccine development and a scientific update for each of these areas is outlined in the report.

So far, there have been 35 studies initiated with the CAVD's Mouse Immunology Laboratory and the Antibody and T-cell Vaccine Immunology Monitoring Consortia; 16 of those studies have been completed and the data has been shared among CAVD researchers. Additionally, 47 articles based on work conducted by CAVD collaborators have so far been published in peer-reviewed journals.

The CAVD supports the goals of the Global HIV Vaccine Enterprise, as described in its Scientific Strategic Plan, which was first proposed in 2003 by a number of HIV researchers and policymakers as a way to promote multidisciplinary and collaborative approaches to generating and testing vaccine candidates. Like the Center for HIV/AIDS Vaccine Immunology—which was established by the National Institute of Allergy and Infectious Diseases in 2005—the CAVD draws together experts from different disciplines to take on specific projects that can inform vaccine discovery. The CAVD was conceived as a translational program that harnesses existing or new science with the objective of developing candidate vaccines to be tested in proof-of-concept clinical trials. It also emphasizes collaboration through the use of standardized reagents and assays, as well as in sharing data as quickly as possible. —*Regina McEnergy*

KAVI Marks 10-year Anniversary

IT WAS 10 YEARS AGO that the Kenya AIDS Vaccine Initiative (KAVI) became involved in the search for an AIDS vaccine. But the seeds of this organization, which is headquartered at the University of Nairobi and was created by local researchers with funding from IAVI and the Medical Research Council's Human Immunology Unit at Oxford University, were planted much earlier. In the early 1980s, a number of Kenyan scientists—in partnership with researchers from the University of Manitoba—started to notice that a small percentage of commercial sex workers remained HIV uninfected over time despite repeat exposure to HIV (see *Individual Armor Against HIV, IAVI Report*, July-Aug. 2008).

Three leading Kenyan scientists involved in this research helped establish KAVI in 1999—Professor Omu Anzala, KAVI's Program Director; Professor Walter Jaoko, Deputy Program Director of KAVI; and the late Professor Job Bwayo, a co-founder of KAVI, who was tragically killed in 2007. “Until KAVI, vaccine research had never really been carried out in this country,” says Anzala. When KAVI was first established, some people were skeptical that an institution of this kind in Kenya would be able to meet the “level and standards” needed to conduct clinical trials, he adds. But Anzala says KAVI has not only met those standards, but raised the bar, both scientifically and ethically.

KAVI has been a productive partner in vaccine research and development, conducting four Phase I trials, as well as a Phase IIa trial of a clade A HIV-DNA/modified vaccinia Ankara prime-boost candidate, all at Kenyatta National Hospital (KNH) in Nairobi. KAVI is also participating in an IAVI-sponsored study known as Protocol G, which is analyzing samples collected from a cohort of HIV-infected individuals to look for broadly neutralizing antibodies against HIV. While a primary goal is testing AIDS vaccine candidates, Anzala says KAVI also has the capacity to test preventive vaccines for malaria and tuberculosis, and he hopes the organization can also broaden its scope to include more basic research.

To mark its 10-year anniversary, KAVI hosted a scientific forum, “Emerging Vaccines: A Public Health Priority,” on March 26. KAVI will also recognize the work of its community stakeholders on World AIDS Vaccine Day, which is observed annually on May 18. —*Regina McEnergy*

Research BRIEFS

CMV-based Vaccine Candidate Protects Macaques from Systemic Infection

AN EXPERIMENTAL AIDS VACCINE consisting of a replicating rhesus cytomegalovirus (CMV) vector carrying several SIV genes protected four of 12 rhesus macaques from systemic infection after repeated low-dose rectal challenge with SIVmac239, a recent study has found (*Nat. Med.* 15, 293, 2009). The study suggests that the vaccine induced effector memory T cells directly in the mucosal tissues, which protected the animals from the challenge virus by keeping it from spreading systemically.

Louis Picker, a professor at Oregon Health & Science University and the lead author of the study, says the premise for this study was that replicating vectors like CMV continuously express antigens and, as a result, induce effector memory cells right at the mucosal sites where the pathogen infects. Within minutes or hours after being presented with an antigen from the challenge virus on the surface of an infected cell, effector memory cells can become CD4⁺ or CD8⁺ T cells and either make cytokines or kill the target cells, Picker says. In contrast, prime-boost vaccine candidates that use non-replicating vectors such as MRKAd5, which was tested in the STEP trial, induce central memory T cells. These cells retreat to inductive sites such as the lymph nodes some time after the antigen goes away. Upon challenge, they have to first migrate to the mucosal tissues, which can take as long as a week—likely too long to keep an infection like SIV or HIV from spreading systemically.

“If you want to have a vaccine that contributes to controlling infection at the very outset of the virus crossing the genital or rectal epithelium, [it has to induce] effector memory cells,” Picker says, adding that to “keep effector memory cells specific for a particular antigen in these sites, [we need to have] antigen around all the time.”

In the study, Picker and his colleagues vaccinated 12 rhesus macaques sequentially with three rhesus CMVs, carrying the SIV genes *gag*, *rev-tat-nef*, and *env*, respectively. Half of the vaccinated macaques also received a boost with a combination of all three vectors. These vaccinated and 16 unvaccinated monkeys then received weekly low-dose intrarectal challenges with SIVmac239.

After 12 challenges, all of the unvaccinated macaques were infected. In contrast, four of the vaccinated macaques were protected from progressive infection. Two of them did not show any measurable virus in plasma even after 13 challenges, and two showed low transient viral levels after the first challenge, but not later. All four are still virus free, Picker says, almost a year after the first challenge.

The four protected macaques showed CD8⁺ T-cell responses specific to two SIV proteins that were not included in the vaccine, showing that they were indeed exposed to the challenge virus. However, CD8⁺ T cell depletion did not result in elevated virus lev-

els, suggesting that CD8⁺ T cells were not what kept their virus levels in check. Also, Picker says, while the study was too small to make a definitive conclusion, the protection status of the vaccinated animals did not correlate with MHC class I variants Mamu-B*08 or B*17, which have been associated with CD8⁺ T cell-mediated control of viral replication—one of four protected animals and five of the eight unprotected animals had either B*08 or B*17. “[The infection] is either controlled by another mechanism or [it] was cleared and the infection is no longer there,” he concludes.

One explanation for how the animals could have cleared the virus is SIV-specific effector memory T cells, which were observed in blood and broncho alveolar lavage of the vaccinated macaques. Picker says the study did not measure these cells in the rectal mucosa because it would have interfered with the experiment, but he suspects they were likely there at the time of challenge, keeping the virus replication below a threshold at which it could sustain itself.

Next, Picker plans to study correlates of local protection in the rectal mucosa. In collaboration with IAVI, he will also be comparing this CMV regimen with combination regimens of CMV/Ad5 and DNA/Ad5. Picker says there are also discussions at IAVI about developing a CMV vector that could be used in humans.

“This is a promising finding, which of course ultimately will have to be confirmed in human clinical trials,” says Stanley Plotkin, a consultant at Sanofi Pasteur. Still, Plotkin says getting FDA approval for a trial with CMV could be a challenge. “They tend to be very risk-averse.”

When it comes to possible human trials, one concern is preexisting immunity, because, according to Picker, virtually everybody in developing countries and half the people in developed countries have been exposed to CMV. However, this study suggests that preexisting immunity may not be an issue. Another concern is whether a replicating vector like CMV will be safe in humans, especially in immune-compromised people. It almost never causes problems in people unless they are immune compromised, Picker says, adding that a human CMV vector would be engineered to have even less pathogenicity.

Replicating vectors are not necessarily the only way to keep antigens around at mucosal sites to induce effector memory cells, Picker says. Another way to achieve this could be an annual shot of perhaps even non-persistent vectors. “The mindset has been you do a prime, you do a boost, and that’s it—the person is supposed to be protected for life,” he says. “But the protection they are going to need I believe is effector memory protection and that’s going to require antigen there frequently, if not all the time.” —*Andreas von Bubnoff*

Microbicide Inhibits Innate Immune Response

A MICROBICIDE CONTAINING the surfactant glycerol monolaurate (GML) protected rhesus macaques from repeat high-dose vaginal challenge with SIVmac239, at least in part by inhibiting the early innate immune response, according to a study led by Ashley Haase, a professor at the University of Minnesota (*Nature* 458, 1034, 2009).

In a previous study, Haase and colleagues treated rhesus macaques once daily for months with K-Y warming gel, which is used as a personal lubricant in humans, either alone or containing GML, and found that neither formulation harmed the vaginal epithelium (*Antimicrob. Agents Chemother.* 52, 4448, 2008).

The researchers then vaginally challenged two GML-treated and two K-Y only treated animals from this safety study with two high-dose challenges of 100,000 infectious doses of SIVmac239, separated by four hours. This challenge leads to infection in at least 90% of animals, Haase says. The gel was applied twice, one hour before each challenge. Two weeks later, one of the two K-Y only animals was infected, while both GML-treated animals were not.

When the same challenge was given to three additional GML-treated and three K-Y only treated control animals from the safety study, one of the control animals was infected by two weeks, and none of the GML-treated animals were infected. After an additional challenge a few weeks later, the remaining two control animals became infected, while all three GML-treated animals remained uninfected. However, Haase says eventually one of the GML-treated animals did become infected five months after the second challenge.

The candidate microbicide PRO 2000 was recently found to reduce the risk of HIV infection by 30% in a Phase IIb trial (see *Canvasing CROI, IAVI Report*, Jan.-Feb. 2009). While the results were not statistically significant, they generated excitement among micro-

bicide researchers. PRO 2000 is thought to work by inhibiting the virus itself, but Haase's study suggests that GML works, at least in part, by inhibiting a chemokine produced by the mucosal epithelium called MIP-3 α , part of the innate immune response. Haase says production of MIP-3 α leads to an influx of more CD4⁺ T cells to the site of infection, providing more target cells for the virus. "Everybody, myself included, thinks of these innate and inflammatory responses as the host responses to prevent and contain infection," he says. "But on balance they do just the opposite—they bring in the fuel."

Within a day after vaginal challenge in Haase's study, researchers observed production of MIP-3 α by the mucosal epithelium. This attracts plasmacytoid dendritic cells, which produce chemokines that in turn attract CD4⁺ T cells. GML-treated monkeys had less MIP-3 α in their vaginal fluid than untreated animals, Haase says, adding that microarray analysis showed a downregulation of MIP-3 α expression in these animals.

GML, found naturally in breast milk, has been widely used by the food and cosmetics industry as an emulsifier, Haase says. Because of its antimicrobial properties, it has also been used in tampons to prevent toxic shock syndrome.

"Only time will tell whether this is a major breakthrough," says Robin Shattock, a professor of cellular and molecular infection at St. George's, University of London, who was not involved in the study. He says it is unclear how often the compound might have to be applied to block infection, and suggests that GML may also have directly inactivated the virus. Haase acknowledges that GML may also work by inhibiting the virus, but says that preliminary observations suggest much lower GML levels in the animals than those at which one would expect to see viral inhibition. —*Andreas von Bubnoff*

Researchers Catch HIV on Film

USING HIGH-SPEED THREE-DIMENSIONAL imaging equipment and an infectious clone of HIV embedded with green fluorescent protein (GFP), researchers were recently able to track and film in real-time the movement of HIV Gag in live CD4⁺ T cells. These movies show what happens when HIV-infected cells collide with uninfected CD4⁺ T cells and convey how rapidly the viral material—with the help of adhesive contacts called virological synapses that are formed at the juncture of CD4⁺ T cells—passes from infected cell to uninfected cell (*Science* 323, 1743, 2009).

Together, virologists at Mount Sinai School of Medicine in New York City, who created the fluorescent HIV clone known as HIV Gag-iGFP, and physicists at the University of California-Davis, who supplied the expertise in high-speed imaging, produced 12 movies. Some depict just a few seconds in the life cycle of the virus, while others—with the help of time-lapsed photography—span several days. Although these short films may not become Hollywood blockbusters, after a week on

YouTube (www.youtube.com/GreenVSLab), one had more than 150,000 hits.

Benjamin Chen, the Mount Sinai virologist who created HIV Gag-iGFP, says a fast video microscope capable of taking three-dimensional images of infected cells every second or so, showed HIV Gag quickly congregates at the virological synapse, forming a button shape, once an infected cell touches an uninfected cell. The footage then shows the viral proteins being ushered into a target cell's endosome, a membrane-bound compartment that many other viruses use to gain entry into cells but which HIV was not thought to favor much. When the HIV Gag-iGFP was compared to an infectious HIV clone without the Env protein, researchers found that this protein is critical for formation of synapses.

The role of cell-associated virus in HIV transmission has long been a mystery. Chen says future vaccine strategies should perhaps look at unique cell-surface Env epitopes that block cell-associated virus from spreading. —*Regina McEnerly*

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