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NHP MEETING 2011

## **MORE MONKEY BUSINESS**

ALSO:

## **The Flu Fighters**

## EDITOR'S LETTER

i

In 2011, the year marking the 30<sup>th</sup> anniversary of AIDS, HIV prevention researchers had cause to reflect, and to celebrate. First came news in May from the landmark HPTN 052 trial, showing that earlier administration of antiretroviral (ARV) therapy to the HIV-infected partners in serodiscordant couples could reduce HIV transmission by 96%. Then, just before this year's International AIDS Conference, results from two trials of pre-exposure prophylaxis (PrEP; the administration of ARVs to uninfected individuals) showed this strategy was 62%-73% effective at preventing HIV infection.

The results from these three studies inspired optimism and may lead to new policy recommendations or guidelines on the use of ARVs to treat HIV infection earlier or prevent it from occurring altogether.

However, with regards to PrEP, the story is far from over. Since the results of these two trials were released, investigators conducting a large, multi-site, efficacy trial known as VOICE, involving approximately 5,000 women, have announced the discontinuation of the arm testing the ARV-based microbicide gel (which was shown to reduce HIV infection rates in the CAPRISA 004 trial by 39%) and the one-drug PrEP arm of the trial, both for futility. Now, only the two-drug oral PrEP arm is continuing. Although the analysis of adherence and other factors will be critical to understanding these results, they have nonetheless raised questions about how well PrEP may work in different populations.

In vaccine research, the biggest news of the year came from the correlates analysis of the RV144 trial in Thailand, the first to show any efficacy (see *A Bangkok Surprise*, *IAVI Report*, Sep.-Oct. 2011). In addition to that, there continues to be incremental progress in elucidating the epitopes targeted by the dozens of broadly neutralizing antibodies researchers have amassed in the past few years (see page 15), and in extracting information from nonhuman primate models of HIV/AIDS (see page 4).

In this issue, we also feature an update from the Keystone Symposium on Malnutrition, Gut-Microbial Interactions and Mucosal Immunity, the first Keystone meeting in India, at which researchers discussed the link between nutrition, gut health, and the immune response to vaccines (see page 12).

While 2011 was certainly eventful, there is still much work to do. The number of new HIV infections last year plateaued, and there were impressive declines in HIV incidence in several sub-Saharan African countries, but at the same time there was a marked increase in infection rates in other parts of the world (see page 15). Progress is afoot, but the science, funding, and commitment to HIV/AIDS prevention remain as important as ever.



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](http://www.iavi.org).

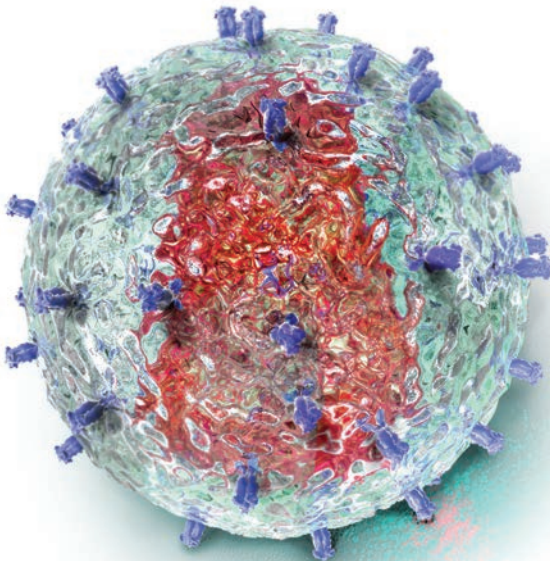
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## [ ON THE COVER ]

The image shows that CD4<sup>+</sup> T cells (red) are quite dense and close to the surface of the thinned squamous vaginal epithelium in a Depo-Provera treated rhesus macaque (nuclei of epithelial cells are blue; green shows autofluorescence). Depo-Provera is a contraceptive that mimics an extreme version of the luteal phase of the menstrual cycle by thinning the vaginal mucosa. The observation that CD4<sup>+</sup> T cells are closer to the surface of the vaginal mucosa in Depo-Provera treated than in untreated rhesus macaques could explain that in humans, Depo-Provera has recently been found to increase the susceptibility to HIV infection in women (*Lancet Infect. Dis.* 12, 19, 2012; see page 6).

*Image courtesy of Ann Carias and Tom Hope of Northwestern University and Ron Veazey of Tulane University.*

# MORE MONKEY *Business*

**Growing attendance at the annual nonhuman primate conference reflects collaboration and the importance of this model for the field**

**By Andreas von Bubnoff**

More and more researchers are becoming interested in monkey business. This year's 29th Annual Symposium on Nonhuman Primate (NHP) Models for AIDS, which took place October 25-28 in Seattle, had about 40% more registrants than last year and double the number of international participants. Autumn in Seattle can be lovely, but conference chair David Anderson, director of the Washington National Primate Research Center, said part of the reason more researchers attended this year's meeting is that NHP research is becoming more collaborative.

An example of this collaboration is the NHP reference transcriptome project that uses next generation sequencing (NGS) to sequence the transcriptomes (the totality of all expressed RNAs) of 15 NHP species. In the past several years, NGS has taken the world of biology by storm, making it possible to understand biological systems in unprecedented detail, and NHP researchers are now using this to their advantage. Other research presented at this year's conference showed how NHPs are used to model almost every aspect of HIV transmission and pathogenesis, allowing researchers to better understand these processes and to address them in humans.

### **Next generation sequencing in NHPs**

Michael Katze, associate director at the Washington National Primate Research Center and a member of the NHP reference transcriptome project's steering committee, gave an update on the current status of the NHP transcriptome project that is sequencing the transcriptomes from 20 different tissues of 15 NHP species. Most of the species being studied are those used in biomedical

experiments, such as the rhesus macaque, although some, such as the gorilla, were included for evolutionary considerations, according to Robert Palermo, a research assistant professor and virologist in Katze's laboratory at the University of Washington who is also involved in the project.

Just 18 months after the project was launched in April 2010, sequencing of the transcriptomes of five species was completed, Katze said, adding that the company Illumina is doing the sequencing work for free. The transcriptomes, Katze said, will tell researchers what sequences are transcribed and therefore likely have a biological function, and will reveal previously unknown RNAs that do not encode proteins and transcripts of previously unknown genes. They will also allow a better annotation of the genome sequences of some of the species such as the draft rhesus macaque genome that was released in 2006.

Others are using NGS to better understand what happens in the host early after infection. Nicholas Maness, currently a scientist in the laboratory of David Watkins at the University of Wisconsin in Madison, used it to study how simian immunodeficiency virus (SIV)-infected host cells transcribe SIV genes in more detail than ever before. The study is among the first that uses NGS to look at viral transcription itself, Maness said. He and his colleagues sequenced all SIV-related transcripts of SIVmac239-infected CD4<sup>+</sup> T cells from Indian rhesus macaques and found several novel protein variants that do not result in any known functional SIV proteins and are even truncated in some cases. It is possible that these "strange" transcripts and proteins do have some still unknown function, but it is also possible that SIV somehow tries to coax the host cell into producing

non-functional proteins to elicit an immune response and thereby distract the immune system from responding to functional SIV proteins. In effect, SIV may be putting up a smoke screen for the immune system, Maness said. He and colleagues showed previously that macaques do make CD8<sup>+</sup> T cell immune responses to some of these seemingly nonfunctional SIV proteins (*J. Virol.* **84**, 11569, 2010).

In another study using NGS, Palermo studied how host cells respond to infection. In the study, male Indian rhesus macaques were challenged with a single, rectal, high dose of SIVmac251. The researchers then used NGS to sequence and quantify all transcripts in the area of the rectum where the challenge virus was applied both three and 12 days after infection, compared with the same area in three of the animals two weeks before they were infected.

Preliminary data from three rhesus macaques, three days after infection when viral replication is still locally restricted only to the challenge compartment in the rectum, confirmed that there already was low-level active viral replication in the tissue. The host tissue gene expression changes at that time suggested that the infection itself is already beginning to cause an inflammatory response in the gut, Palermo said. "It was really, really surprising that you should see evidence of inflammation so early."

The other surprising observation at day three, he said, was downregulation of genes that are involved in tight junctions, structures that hold the epithelial cells of the rectum together. Such changes could increase the permeability of the gut epithelium, allowing the virus to spread to the interior gut-associated lymphoid tissue. These changes could also be early indications of the leaky gut that enables gut bacteria in chronically infected animals to leave the gut, which is thought to be one cause for chronic inflammation in SIV-infected animals and HIV-infected people. There was also an increase in collagen gene expression, suggesting the onset of fibrotic tissue damage that is usually caused by long-term inflammation of tissues. Palermo believes these are the earliest observations of changes in collagen or tight junctions in the gut after infection.

Data from three animals at day 12, when the virus has already spread to blood and all of the lymphoid tissue in the rectum and when the animals are probably close to their peak plasma viremia, showed a very different picture. These animals had a strong type I and II interferon antiviral innate immune response. Next, Palermo wants to analyze animals just one or two days after infection to see if the immune response already provides some local control over the virus this early after infection.

## Characterizing the host

Because host factors that affect viral replication can affect the outcome of challenge experiments, researchers are trying to better characterize such factors in different NHP species that are used for such experiments.

For example, the restriction factor TRIM5 $\alpha$  can restrict SIV replication in host cells. It recognizes and binds the viral capsid as it enters the cell and thereby restricts infection in cells from many monkey species including rhesus macaques. TRIM5 $\alpha$  could therefore affect the outcome of challenge experiments because it might give the impression that the animals were protected.

Another restriction factor, TRIMCyp, is related to TRIM5 $\alpha$  but has cyclophilin A as the capsid binding domain. TRIMCyp has been found in four species of Asian macaques—it was first described in cynomolgus, pigtail, and rhesus macaques in 2008, and in northern pigtailed macaques in 2009, according to Elizabeth Dietrich, a graduate student in the laboratory of Shiu-Lok Hu at the University of Washington. Not much is known about prevalence, diversity, and restriction activity among the cynomolgus macaque population, but Dietrich and her colleagues are studying which versions of TRIMCyp can be found in cynomolgus macaques from different geographical areas, and how different versions of TRIMCyp can affect the susceptibility of cultured cells to infection with HIV-1 and HIV-2 (*J. Virol.* **85**, 9956, 2011).

She found that TRIMCyp is quite common in cynomolgus macaques from Indonesia and the Philippines, but is relatively rare in animals from mainland Indochina, and apparently absent in animals from Mauritius.

Three different TRIMCyp variants were identified in cynomolgus macaques. Expressing each of these variants in a cultured cat cell line (which does not express its own TRIM5 $\alpha$  or TRIMCyp) either restricts HIV-1 infection, HIV-2 infection, or infection with both HIV-1 and HIV-2, depending on changes of the amino acids in just two positions of the cyclophilin A domain. Preliminary results suggest that changes in these same two amino acids also affect susceptibility to infection with SIVsmE041, suggesting that differences in TRIMCyp may also affect how NHP species such as cynomolgus macaques behave in SIV challenge experiments.

While all three variants are found in cynomolgus macaques, only one has so far been found in rhesus and pigtail macaques, Dietrich said, suggesting that cynomolgus macaques are the most diverse

macaque species described so far with respect to the variability of their TRIMCyp restriction factors.

### Modeling human transmission

One big advantage of NHPs is that they can be used as a model for HIV transmission. Thomas Hope, professor of cell and molecular biology at Northwestern University, is using NHPs to study how fluorescently labeled HIV particles enter the mucosal barrier of the female macaque reproductive tract, and how this varies during the menstrual cycle. “[This is] becoming an interesting and important topic,” Hope said.

He studied HIV entry into the vagina of macaques that had been treated with Depo-Provera, a contraceptive that mimics an extreme version of the luteal phase of the menstrual cycle by thinning the vaginal mucosa and has recently been shown to double the susceptibility to HIV infection in women (*Lancet Infect. Dis.* 12, 19, 2012). He found that in Depo-Provera treated animals, more T cells came close to the surface of the vaginal mucosa, possibly explaining why Depo-Provera increases transmission (see cover image).

Hope has also been studying virus penetration in pigtail macaques at different points of the menstrual cycle. While the study is still blinded, there is considerable variation, similar to the difference between animals that were treated with Depo-Provera and untreated animals, he said.

Different stages of the menstrual cycle may also influence the antiviral activity of vaginal gels containing the antiretroviral (ARV) tenofovir, according to Charles Dobard of the US Centers for Disease Control and Prevention (CDC). Dobard reported that in pigtail macaques, the concentration of the active metabolite of tenofovir in vaginal lymphocytes was about five times higher during the luteal phase (when the vaginal epithelium is thinnest and the susceptibility to HIV infection is highest) than in the follicular phase of the menstrual cycle. The tenofovir levels in plasma were also higher in the luteal phase.

“The good thing is that we see more absorption when there is more thinning [of the epithelium], so with the higher susceptibility to infection there is also more drug going in,” said Walid Heneine of the CDC, who led the study, adding that knowing how absorption of ARVs changes during the menstrual cycle is important when interpreting the results of clinical trials, such as the recent CAPRISA 004 trial, which showed for the first time that a microbicide gel containing 1% tenofovir was able to reduce HIV incidence by 39% in a cohort of South African women. Most of the women in that trial were on

Depo-Provera, Heneine said, so “you would think that they were absorbing the drug very well vaginally.” Still, researchers don’t know if the variation in absorption of ARVs during the menstrual cycle in women is the same as in NHPs. “We have to see to what extent this happens in women,” he added.

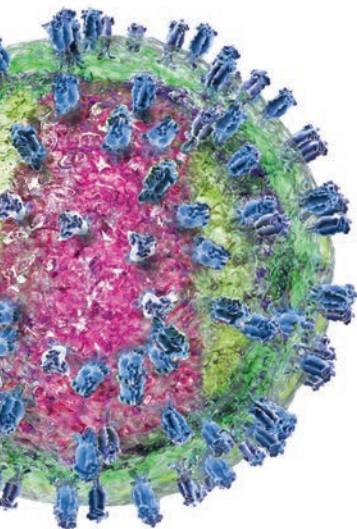
### Reducing viral reservoirs

While highly active antiretroviral therapy (HAART) can lead to a drop in viral loads to undetectable levels in a majority of HIV-infected individuals, eliminating the remaining viral reservoirs is still one of the biggest challenges to curing HIV. Deborah Fuller, an associate professor in the department of microbiology at the University of Washington, reported that a therapeutic vaccine can further reduce residual virus levels in ARV-treated rhesus macaques and also suppress the virus even after ARV treatment is stopped.

In the study, Fuller and colleagues intravenously infected rhesus macaques with SIVdeltaB670. Six weeks later, when the animals had high viral loads, they were treated with the ARVs tenofovir and Kaletra, resulting in viral loads of about 10,000 copies per ml of blood. Because the animals in the study were not given HAART, there wasn’t as strong a reduction in viral load as seen in humans on HAART. Rather, the study design was more similar to humans who are not responding very well to drug therapy. Animals that didn’t show at least a 10-fold reduction in viral load in response to therapy were excluded, Fuller said.

Twelve weeks after starting ARV treatment, animals that responded to the drugs were vaccinated with six monthly doses of an SIV DNA vaccine that expressed Gag, Reverse Transcriptase, Nef, and Env that were almost identical to SIVmac239. The vaccine was administered into the epidermis of the skin with a gene gun. This type of delivery elicits a mucosal immune response in the gut. To further augment mucosal immune responses in the gut, the vaccine also contained DNA encoding the heat labile enterotoxin from *E. coli* (LT), a mucosal adjuvant. Induction of mucosal immune responses in the gut was important because the gut harbors a viral reservoir that cannot be eliminated with drug therapy, Fuller said.

While the animals were on ARVs, the LT-adjuvanted vaccine further lowered the average viral load of the monkeys to about 100 copies per ml of blood. When ARV treatment was stopped eight weeks after the final vaccination, the mean viral load did not rebound in the group immunized with the LT-adjuvanted vaccine, whereas in the unvaccinated ani-



imals, the mean viral load rebounded. As a result, six months after stopping ARVs, the vaccinated animals had an almost five log lower mean viral load compared with the unvaccinated animals. “Nobody has ever been able to achieve that level of virus suppression with a therapeutic vaccine before,” Fuller said. Ten and a half months after stopping the ARVs, all seven vaccinated animals still showed no CD4<sup>+</sup> T cell decline and were healthy, whereas five of the six unvaccinated animals had progressed to AIDS.

Fuller said the study also showed for the first time that compared with the unvaccinated controls, the therapeutic vaccine was able to achieve a significant reduction in the amount of virus in the viral reservoirs in the gut and other tissues.

Steven Deeks, a professor of medicine at the University of California in San Francisco, said that one reason that even people on HAART still have an about ten year shorter life expectancy than uninfected people is that they show residual inflammation, probably because of remaining virus in tissue reservoirs. He said Fuller’s study shows that it might be possible to use a therapeutic vaccine to treat this residual inflammation in people who are on HAART. “It’s one of the few studies I have seen in which you can give an intervention that affects the size of the reservoir,” Deeks said.

## Modeling STEP

NHP models are also being used to better understand the results of recent clinical trials of HIV vaccine candidates. Irene Bukh, a graduate student in the lab of Michael Betts at the University of Pennsylvania School of Medicine, is using NHPs to try to address why in the STEP trial, Merck’s MRKAd5 vaccine candidate not only failed to protect from HIV infection but in some vaccinees with previous adenovirus serotype 5 (Ad5) immunity actually increased the risk of HIV infection. One hypothesis has been that the vaccine may have increased HIV infection risk by increasing the number of activated CD4<sup>+</sup> T cells, targets for HIV infection, in the gut or rectum.

To test this hypothesis, Bukh and colleagues tried to replicate the STEP trial in rhesus macaques. However, because human Ad5 doesn’t naturally infect rhesus macaques, researchers instead used a replication incompetent simian Ad7 (SAd7) vector derived from a naturally occurring adenovirus that infects rhesus macaques. About half of the rhesus macaques used in the experiments had antibodies to SAd7 in their blood, which is similar to the percentage of pre-existing immunity to Ad5 in humans in the US, one of the areas where the STEP trial was

conducted, Bukh said. Also, unlike the vaccine used in the STEP trial, the SAd7 vector carried the spike protein from the human SARS virus, not SIV proteins. “We were just looking for the responsiveness to adenovirus,” she said.

Bukh and colleagues vaccinated 12 rhesus macaques three times intramuscularly with this SAd7 vector, with a prime at week 0 and two boosts at week 17 and 31. This too was different from the STEP trial where the three intramuscular vaccinations were done at weeks 0, 4, and 26. They also included five animals vaccinated with human Ad5 as controls.

Researchers extracted CD4<sup>+</sup> T cells from peripheral blood and rectal biopsies before and several times after each of the three vaccinations. They then stimulated them *in vitro* by adding the same replication incompetent SAd7 vector they used for vaccination to reactivate any adenovirus-specific CD4<sup>+</sup> T cells that were still around from previous exposures to SAd7, turning them into activated CD4<sup>+</sup> T cells, which are the targets for SIV and HIV.

They found that this treatment could stimulate a greater fraction of CD4<sup>+</sup> T cells taken from the rectum than from blood of the vaccinated animals. The animals vaccinated with human Ad5 and animals without previous SAd7 antibodies in their blood also showed a larger fraction of Ad7 CD4<sup>+</sup> T cells that could be activated in their rectum than in blood after SAd7 vaccination, suggesting that even vaccination with, or exposure to, adenoviruses other than SAd7 might increase the animal’s fraction of CD4<sup>+</sup> cells in the gut that can be activated by SAd7 vaccination.

Bukh said the SAd7 vaccination likely reactivates memory CD4<sup>+</sup> T cells from previous adenovirus infections that then home to the place where they got activated during the initial infection. Because many adenovirus infections target the gut, the memory cells will typically home to the gut, Bukh said, explaining why the Ad7 vaccinated animals had a higher percentage of activated Ad7-specific CD4<sup>+</sup> T cells in the rectum than in blood.

This means that as a result of the vaccination, “there might be a redistribution of the Ad-specific CD4<sup>+</sup> T cells from the peripheral blood to the rectal lamina propria,” Bukh said. “If you are exposed [to] HIV or SIV at that point, you could have a higher likelihood of infection.”

To see if animals with a larger fraction of Ad7-specific CD4<sup>+</sup> T cells in the rectum are indeed more likely to get infected, Bukh and colleagues next plan to vaccinate another group of macaques in a similar way and then intra-rectally challenge them with SIVmac239 or 251. ■

## [ HOW HSV-2 INFECTION INCREASES HIV-1 INFECTION RISK ]

Herpes simplex virus (HSV)-2 infection, which is the cause of genital herpes, is known to increase the risk of HIV infection, in part because the lesions and inflammation it causes increase the number of HIV target cells. This suggests that treating HSV-2 infection should reduce the increased risk of HIV infection, but a randomized trial called HPTN 039 found that a twice-daily 400 mg dose of acyclovir to treat HSV-2 infection did not reduce HIV infection risk (see *Clues from CROI, IAVI Report*, Jan.-Feb. 2008).

At the Annual Symposium on Non-human Primate Models for AIDS, Elena Martinelli, a scientist at the Population Council in New York City, reported on experiments that suggest a possible explanation of how HSV-2 infection could increase susceptibility to HIV infection, even in the absence of the lesions or inflammation caused by HSV-2.

Her study was based on previous findings that HIV and SIV bind to a receptor called  $\alpha 4\beta 7$  on CD4<sup>+</sup> T cells and that CD4<sup>+</sup> T cells that express very high levels of this receptor are more susceptible to HIV and SIV infection and produce more virus.

Martinelli found that rectally infecting macaques with HSV-2 leads to an increased percentage of CD4<sup>+</sup> T cells with high  $\alpha 4\beta 7$  expression in their blood and rectal mucosa, suggesting that they might be more susceptible to SIV infection. She and her colleagues then showed that infecting dendritic cells with HSV-2 *in vitro* caused the dendritic cells to secrete the vitamin A derivative retinoic acid, which in turn led to increased expression of the  $\alpha 4\beta 7$  receptor on CD4<sup>+</sup> T cells and a concomitant increase in HIV replication in the CD4<sup>+</sup> T cells.

The work suggests that blocking the  $\alpha 4\beta 7$  receptor might reduce the increased susceptibility to HIV infection in patients that are coinfecting with HSV-2 (*PLoS Pathog.* 7, e1002109, 2011). —AvB

# THE FLU *Fighters*

The influenza vaccine pipeline is diverse and crowded with candidates that have the potential to transform the field

By Regina McEnergy

In what could be described as a 21st century Flu Rush, influenza researchers have been aggressively pursuing dozens of candidates over the past decade that could possibly work better, last longer, and be easier and quicker to manufacture than the current crop of nearly 30 licensed influenza vaccines.

The impetus for this surge in vaccine development is two-fold: to reduce the global impact of seasonal influenza, which results in an estimated three to five million severe illnesses and 25,000-50,000 deaths annually, and to dramatically improve emergency preparedness for the unpredictable nature and sometimes tsunami-like strength of pandemic influenza strains.

The hemagglutinin (HA) molecule that juts out from the surface of the influenza virus (see image, page 9) continually mutates, requiring vaccine developers to update the seasonal influenza vaccines annually to adapt the vaccine to the two to three dominant strains they predict will be in circulation that year. Therefore, finding better approaches is a global public health priority.

Whether scientists will succeed in building a better flu trap remains to be seen, but there are certainly plenty of contestants vying for the prize. Eighty-five candidates are in various stages of preclinical or clinical development (see Figure 1, page 10). Ten of the vaccine candidates are considered universal vaccine candidates and are designed to protect more people against a broader range of influenza viruses for a longer period of

time. More than half of those in all stages of development employ innovative strategies not yet available in any licensed seasonal or pandemic influenza vaccine. And at least 60 of the 85 candidates are in various stages of clinical development—about double the number of AIDS vaccine candidates in clinical testing.

Most of the candidates (72%) are in early stages of clinical development, so odds are many will fail. Still, vaccine manufacturers find the upsurge in research exciting. “Influenza used to be the sleepy backwater town of vaccine manufacturing,” says Alan Shaw, the chief scientific officer of VaxInnate, a New Jersey-based biotech whose seasonal virus-like particle (VLP) and universal vaccine candidates have both been tested in Phase II trials. “It’s really remarkable what is happening.”

Frank Arnold, senior program manager of the influenza division at the Biomedical Advanced Research and Development Authority (BARDA), a branch of the US Department of Health and Human Services (HHS), agrees. He says while vaccine manufacturers have long recognized the need to advance the influenza vaccine technology, the swine flu H1N1 virus that appeared in 2009 and triggered a global pandemic really solidified why this goal is so important. “It took manufacturers six months to get the product out the door,” notes Arnold. “We missed the first two waves and if it was a 1918-like epidemic it would have been a disaster.”



Arnold's reference to the novel 1918 avian influenza strain that sparked the mother-of-all influenza pandemics is every public health person's worst nightmare—a lethal, fast-spreading H1N1 strain that claimed 50 million lives between 1918 and 1920.

When a novel, highly pathogenic avian strain of H5N1 was first detected in humans in 1997 in Hong Kong, some feared a repeat, but the virus, while claiming 300 of its 500 victims, wasn't transmitted efficiently from person to person and in nearly every case appeared to have been transmitted through contact with infected poultry rather than through human contact. More worrisome though, says Arnold, would be if the circulating H5N1 strain, largely confined to a handful of countries in Asia, was able to genetically re-assort with a pandemic strain like H1N1 to form a new strain that is both swift and violent. "You would have massive issues," he says.

This fear of being blindsided by a crippling pandemic drove HHS in 2004, mostly through the work of BARDA, to devote US\$2.2 billion to the development of vaccines, antiviral drugs, diagnostics, respirators, and ventilators to prevent and treat pandemic influenza, with about \$1.8 billion appropriated for vaccine candidates employing cell-based technologies, antigen-sparing adjuvants, and recombinant and molecular technologies. This financial windfall drove many large pharmaceutical companies and smaller biotech to create or expand their influenza vaccine research and development programs and is the primary reason why the pipeline is now so active.

The global push to make influenza vaccination more affordable in developing countries is also contributing to the crowded pipeline. Developing countries can't afford to buy flu vaccines, so they are taking their own candidates through the development process using the egg-based technology that is now used to make most seasonal flu vaccines. Arnold says the difference in cost per shot—\$5 vs. 50 cents—says it all. "This is the cheapest and fastest route for the developing world."

## A universal solution

Not surprisingly, a universal vaccine candidate capable of providing protection against all influenza type A strains, which comprise 66% of all human strains, would be the ultimate achievement, though the approach, for now, is considered a long shot. At a World Health Organization meeting in November, researchers and public

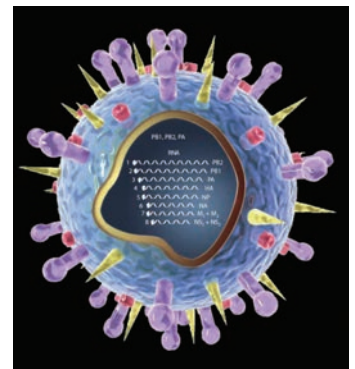
health leaders discussed the possibility of generating universal immunity against influenza, and the panel is expected to release a position paper soon summarizing how researchers are trying to achieve this goal.

Though not as variable a virus as HIV, influenza presents significant challenges for universal vaccination because of the slight but rapid antigenic shifts on the virus' surface from season to season. Developers of universal vaccine candidates are attempting to overcome this obstacle by targeting the more conserved areas of the HA molecule or influenza's other proteins. So far, this approach has provided mixed results.

One candidate, a recombinant fusion protein called VAX102 developed by VaxInnate, targets the matrix protein 2 extracellular domain (M2e), a short, stubby surface protein that is more conserved than HA across influenza subtypes, but which doesn't induce much of an immune response on its own. VaxInnate's approach genetically fuses the ectodomain located on the outer region of the M2e protein's cell membrane to bacterial flagellin, the long, hair-like tails that enable bacteria to swim, and which interact with toll-like receptors (TLRs), a class of proteins that play a key role in innate immunity. A Phase I study in 60 adults showed that VAX102 alone induced a strong immune response to the M2e protein when it was joined with the TLR agonist (*Vaccine*, 29, 5145, 2011), and when tested in combination with a trivalent seasonal flu vaccine it appeared it might enhance the immune responses to the standard vaccine, as well as add a secondary immunity to the M2e component (*PLoS One* 5, e14442, 2010). However, VaxInnate's VAX102 program was put on hold over problems with the M2e protein sequence, putting the whole concept of developing this universal influenza vaccine candidate in doubt.

The University of Oxford has adopted another strategy with its universal vaccine candidate, a recombinant modified vaccinia Ankara (MVA) viral vector vaccine candidate containing sequences that code for matrix 1 (M1) and nucleoprotein (NP), internal proteins that are highly conserved and associated with cell-mediated immunity.

Sarah Gilbert, who heads up the project at Oxford's Jenner Institute, recently tested the MVA-NP-M1 vaccine candidate in a dose-escalation Phase I study involving 28 men and women ages 18-50 from the UK and found the vaccine boosted interferon- $\gamma$  secreting, antigen-specific



An illustration of the influenza virion. Shown on the surface are three proteins: hemagglutinin (in purple), neuraminidase (in yellow), and matrix protein 2 extracellular domain (in red).

CD8<sup>+</sup> and CD4<sup>+</sup> T cells (*Clin Infect Dis.* 52, 1, 2011). Gilbert also recently completed a Phase IIa study in 22 men and women from the UK, half of whom received the MVA vaccine candidate and half who received no vaccine. The volunteers were then quarantined for 7-10 days and challenged nasally with an A/Wisconsin strain of the H3N2 flu strain isolated in 2005 that is still in circulation. Gilbert described the results of the study in vague terms at the Influenza Congress USA conference Nov. 8-10 in Virginia, saying fewer vaccinees developed influenza or moderate-to-severe influenza symptoms compared to the controls.

Researchers are also attempting to build a universal influenza vaccine candidate by focusing on the stalk of the HA protein, rather than its mushroom-like head targeted by existing flu vaccines. Peter Palese, the Horace W. Goldsmith Professor and chair of the department of microbiology at Mt. Sinai School of Medicine in New York, is one of several researchers trying to generate cross-reactive stalk-specific antibodies to flu.

Palese demonstrated in mice that he could elicit the production of a handful of rare broadly neutralizing monoclonal antibodies against an array of H3N2 viruses responsible for most of the influenza morbidity and mortality in the last 43 years. His lab determined that the antibodies worked by inhibiting viral fusion, and identified the binding site of one of the monoclonal antibodies on the

stalk of the HA as a continuous region that was 100% conserved between the H3 viruses used in the study (*PLoS Pathog.* 6, e1000796, 2010).

In a separate experiment, Palese’s laboratory developed a method of chemically treating purified influenza virus to behead the HA protein. They then immunized mice with the truncated HA and found that the headless HA was sufficiently immunogenic to induce antibodies against multiple subtypes, and though it was not as protective as the full-length HA immunogen used in conventional vaccines, it protected against lethal challenge (*mBio* 1, e00018-10, 2010). “The next step is trying to get this vaccine into people,” says Palese.

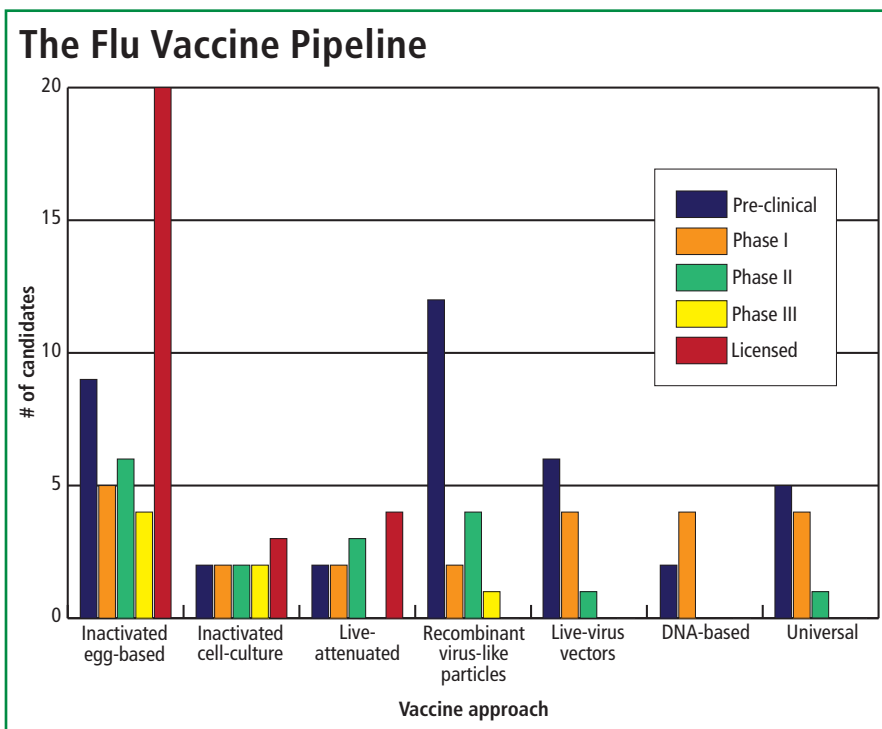
However, it remains to be seen if it is possible to design immunogens that can elicit these stalk-specific antibodies, says John Treanor, head of the infectious diseases division at the University of Rochester Medical Center and a longtime influenza vaccine researcher who is not involved in the Palese project. “It’s absolutely possible to get the immune system to make those antibodies, but there are still lots of obstacles.”

**Faster production**

Another goal, now within closer reach, is being able to manufacture vaccines for seasonal and pandemic flu more quickly. Since the start of the Cold War, pharmaceutical manufacturers have used fertilized chicken eggs to grow live strains of three dominant influenza viruses, which are then inactivated and inserted into a vaccine.

Global health authorities and flu vaccine manufacturers have remained loyal to the egg because it is cheap and safe, and because the vaccines produced using this method have, for the most part, been moderately effective in preventing seasonal influenza. But the 8-10 months required to produce a typical trivalent seasonal vaccine using this approach can lead to delays in seasonal vaccine production. Even more problematic is when a single-strain or monovalent vaccine is suddenly needed to attack pandemic flu strains, as was the case in 2009 when the pandemic H1N1 virus surfaced in April of that year. The novel strain was first detected in Mexico and the US, and though the percentage of those who became ill or died from causes related to the H1N1 pandemic turned out to be far lower than the previous three pandemics—only 18,000 people are estimated to have died from it worldwide—it still raced around the globe. By the time a vaccine to combat the pandemic was ready for distribution, it was October

FIGURE 1



Source: Biomedical Advanced Research and Development Authority

and the second wave of cases was already peaking.

Global health leaders and flu manufacturers recognize the limitations of growing the seasonal flu vaccine in chicken eggs, just as they are aware that the seasonal shots, now available at so many different venues that even protestors at Zuccotti Park lined up to get them during the New York City Occupy Wall Street protest, have not been well studied in high-risk populations. This point was captured in a recently published meta-analysis of 31 influenza vaccine studies conducted over the past decade. The study noted that “evidence for consistent high-level protection is elusive for the present generation of vaccines, especially in individuals at risk of medical complications or those aged 65 years or older,” (*Lancet Infect. Dis.* 2011, doi: 10.1016/S1473-3099(11)70295-X).

But researchers are optimistic about overcoming these challenges with new approaches. The next-generation influenza vaccine candidate closest to regulatory approval is a recombinant vaccine that is grown in insect cells. The trivalent vaccine candidate representing strains of H1, H3, and B influenza strains, known as FluBlok, is made by cloning HA genes from target flu viruses and splicing them into baculoviruses that are then used to infect ovary cells of caterpillars. Baculovirus is found on vegetables and infects some insects, but not mammals, birds, fish, or other species. This process allows the vaccine to be produced in a matter of weeks, says Daniel Adams, executive chairman and global head of business development at Protein Sciences, the developer of FluBlok.

A randomized placebo-controlled study of nearly 5,000 adults ages 18-49 conducted during the 2007-2008 influenza season found FluBlok was 45% effective in preventing culture-confirmed influenza meeting the US Centers for Disease Control and Prevention case definition for influenza-like illness, despite significant antigenic mismatch between the vaccine antigens and circulating viruses (*Vaccine* 29, 7733, 2011). The study was led by Treanor, who is also a medical advisor at Protein Sciences.

In a separate study of 601 healthy adults ages 50-65 comparing FluBlok with a trivalent seasonal influenza vaccine, FluBlok was found to be safe and immunogenic, but those who received FluBlok had a higher seroconversion rate with the H3N2 strain than those in the seasonal vaccine group (*Vaccine* 29, 2272, 2011). The company has also developed a pandemic influenza vaccine candidate called PanBlok against an H5N1 strain using the same baculovirus platform and tested it in Phase I and II trials.

Still, it's been an uphill battle to licensure. Two years ago, an advisory panel declined to recommend to the US Food and Drug Administration (FDA) that Protein Sciences be granted a license for FluBlok because of insufficient safety data, which the company disputed. The FDA sided with Protein Sciences and is not requiring the company to conduct any additional larger trials or resubmit its application. Adams says Protein Sciences is hoping for FDA approval in early 2012.

## Cell-based technology

Another non-egg-based approach that has garnered significant public and private investment is cell-based technology, which involves growing the influenza virus in mammalian cells, usually kidney cells. The virus is injected into the cells where it multiplies, then the cells' outer walls are removed, harvested, purified, and inactivated.

Because it is not made inside a chicken egg, it is safe for people with egg allergies, but the entire production is only slightly shorter than egg-based methods and is not without risk. The amount of vaccine produced depends upon the strain—some grow faster than others—making it difficult to accurately predict how long it will take to meet inventory. Also, the manufacturing process requires expensive biosafety measures to ensure that the vaccine stock isn't contaminated.

Regulators from Europe and India have licensed three different cell-based vaccines, and the FDA could be ruling soon on two others developed by Baxter Sciences and Novartis Vaccines & Diagnostics, the maker of the European flu vaccines.

The development of cell-based technology for influenza vaccination has been inching forward since the 1990s, but recent BARDA investments have greatly accelerated the process. Novartis, for instance, was awarded a \$487 million, multi-year contract from HHS two years ago to construct the first facility in the US for cell-based seasonal and pandemic influenza vaccines. The contract required that Novartis pay 60% of the construction costs of the \$1 billion North Carolina complex, which was completed this year and is designed to produce 150 million doses of monovalent vaccine or 50 million doses of trivalent vaccine within six months.

BARDA had similar partnerships with GlaxoSmithKline Biologicals, Sanofi Pasteur, MedImmune, and CSL Biotherapies to increase US pandemic vaccine manufacturing capacity. “We're reaching a point where we can pick that fruit,” says Robert Huebner, BARDA's deputy director of the influenza division. ■

# A GUT RESPONSE *to Vaccines*

At the first Keystone meeting in India, researchers discussed how malnutrition and gut health affect the immune system

By Andreas von Bubnoff

A healthy gut and good nutrition are important for a healthy immune system and a good immune response to vaccination. This is likely the reason that oral vaccines have been found to be less efficient in malnourished children with a disordered gut. Still, surprisingly little is known about how nutrition and gut health affect the mucosal immune system in the digestive tract.

The Keystone Symposium on Malnutrition, Gut-Microbial Interactions and Mucosal Immunity to Vaccines, which took place in New Delhi Nov. 7-11, addressed what is known about these issues and how to develop interventions that could improve the gut immunity and response to oral vaccines among children in developing countries.

## Vaccine responses

While children in developing countries have a poorer response to oral vaccines, such as oral polio or rotavirus vaccine, than children in developed countries, their response to vaccines that are administered systemically by injection, such as the measles vaccine, is much less impaired.

Gagandeep Kang, professor and head of the Wellcome Trust Research Laboratory at the Christian Medical College in Vellore, India, and one of the organizers of the conference, compared the efficacy of oral rotavirus vaccines in children from developing and developed countries. There are currently two licensed oral rotavirus vaccines that both contain live-attenuated viruses—GlaxoSmithKline's (GSK's) Rotarix and Merck's RotaTeq.

Kang said these vaccines were developed after a study in Mexican children showed that after two rotavirus infections, children had developed complete protection from severe diarrhea caused by

subsequent rotavirus infections (*N. Engl. J. Med.* 335, 1022, 1996). However, when Kang and colleagues did a similar study in children in Vellore, India, they found that after two infections, only 59% of the children were protected from severe rotavirus diarrhea, and only 79% were protected after three infections (*N. Engl. J. Med.* 365, 337, 2011). The lower level of protection in the Indian children could be partly because they got infected at a younger age when their immune systems were still immature, Kang said. Another possible reason is that they might have received a high level of maternal antibodies, which could have neutralized the incoming virus, inhibiting the development of an immune response.

Based on her results, Kang and colleagues have done modeling studies that suggest the existing oral rotavirus vaccines would have less than 50% efficacy in India, similar to the efficacy for these vaccines in other developing countries. Studies published last year showed that the efficacy of Rotarix was 77% in South Africa and 50% in Malawi (*N. Engl. J. Med.* 362, 289, 2010), and the efficacy of RotaTeq was between 40% and 50% in Ghana, Kenya, Mali, Bangladesh, and Vietnam (*Lancet* 376, 606, 2010; *Lancet* 376, 615, 2010). In contrast, these vaccines have at least 90% efficacy in developed countries.

However, the lower efficacy of the rotavirus vaccines in countries such as India doesn't mean they shouldn't be used there, Kang said. "Even at current levels of efficacy, you will save 50,000 children every year." At the same time, she added, we also need to improve their efficacy or come up with alternate vaccines that are more efficacious in developing countries.

One option is to develop injectable rotavirus vaccines. In the US, several injectable candidates

are currently in preclinical studies, Kang said. Another option is to develop cheaper oral vaccines because such vaccines are easier to deliver, particularly in very young children, making it easier to deliver several doses to improve efficacy, Kang said. Indian scientists are currently developing a cheaper version of a new oral live-attenuated rotavirus vaccine. Kang is involved in a randomized Phase III clinical trial that is testing this vaccine in children in Delhi, Vellore, and Pune. She said an Indian vaccine would most likely cost less than US\$1 per dose, compared with about \$20-25 per dose that it costs to buy the existing oral vaccines.

### Why oral vaccines perform worse

Many children in developing countries have a disordered gut, which contributes to their poor response to oral vaccines, Kang said. They suffer from a complex syndrome that involves gut inflammation, altered gut microbiome, intestinal infections with several putative pathogens, impaired nutrition, and impaired growth, according to Chris Wilson, director of discovery at the Bill & Melinda Gates Foundation. But understanding the underlying causes for this syndrome and why vaccines don't work in this environment isn't easy, he said.

Another problem is that malnutrition and immune system dysfunction likely exacerbate each other, Kang said. Malnutrition seems to cause the gut to fail to maintain its immune and barrier function from pathogens, causing diarrhea and infections. This causes further gut damage and affects the gut's ability to absorb nutrients, which makes the malnutrition worse, Kang said.

Also, it is difficult to study what happens in the gut because doing so typically requires taking biopsies. Therefore, measuring processes in the gut is only possible indirectly, using markers in the blood or stool, such as serum or fecal immunoglobulin (Ig) A levels, Kang said. To address this issue, the Gates Foundation announced US\$9 million in grants at the meeting to support research to find better non-invasive biomarkers to assess gut function and health.

Despite these challenges, researchers are beginning to understand what happens in the gut of malnourished children. William Petri, a professor of medicine at the University of Virginia, and his colleagues found that in a group of three-year-old children from Dhaka, Bangladesh, the children with more stunted growth were less likely to respond to oral polio vaccine (OPV), suggesting a possible role of malnutrition. They also found that these children more often have antibodies against bacterial endotoxins, suggesting that their

guts are damaged and bacteria are leaking out.

Exposure to endotoxins may be the reason why Evan Newell, a research associate in Mark Davis's lab at Stanford University, and colleagues found evidence of chronic inflammation in children from this group who failed to respond to OPV. Newell and colleagues studied a subgroup of 40 of the children studied by Petri, six of whom didn't develop antibodies to polio after the vaccination. To find out why, they stimulated their white blood cells with inflammatory and anti-inflammatory cytokines that modulate the immune response. They then measured how well the cells could respond to the stimulation by measuring the activation of cytokine receptors. They found that half of the children didn't respond well to the cytokines, including the six that hadn't responded to OPV. Further measurements showed that the white blood cells that failed to respond to the cytokines in the children that didn't respond to OPV also had increased expression of inflammatory cytokine genes.

Together, this suggests that chronic inflammation in these children constantly exposes their immune cells to cytokines, eventually desensitizing them so that they can't respond to cytokines or vaccines anymore. "They are just kind of burned out," Newell said.

Petri said this suggests that in the children who failed to respond to OPV, bacteria are leaking out of the gut and induce an overstimulation of the immune system, which paradoxically resulted in suppression of the response to the vaccine. "[It] supports this idea that chronic inflammation in children, perhaps through exposure to bacterial endotoxin in the gut, is causing these different immune cell populations to be non-responsive to cytokine stimulation," Petri said.

The leaky gut comes from a condition called tropical enteropathy, he added, where the gut is so inflamed that the gut villi fuse together. It is thought to be caused by the onslaught of intestinal infections many children in the developing world are exposed to.

### Directing immune responses to the gut

One way to address the underperformance of orally administered enteric vaccines in developing countries is to deliver them through different routes. Conventional intramuscular or subcutaneous immunizations often only induce weak immune responses in the gut, and therefore protect only weakly against gut infections. However, researchers are starting to modify injected vaccines so that they can induce immune responses in

### [ Tregs IN THE GUT ]

Per Brandtzaeg, a professor of medicine at the Oslo University Hospital, provided another possible consequence of the overstimulation of the mucosal immune system in the gut of malnourished children in developing countries. He said that too many regulatory T cells (Tregs) might be generated locally in the gut mucosa of such children. This may explain why their immune response to oral vaccines is suppressed.

Hints that this might be the case come from observations that parasitic infections can result in an excess number of Tregs. Brandtzaeg mentioned a study of a patient who had a parasite infestation that resulted in a large number of Tregs in the gut mucosa. Treatment of the parasitic infestation removed the parasites, but also markedly reduced the number of the patient's Tregs, and the patient developed an over-stimulated local immune response with the features of inflammatory bowel disease.

This suggests that the Tregs induced by the parasite kept the inflammatory bowel disease under control, Brandtzaeg said. If there is a similar expansion of Tregs to dampen the tropical enteropathy in the gut of malnourished children in developing countries, "it could actually dampen the immune system to an extent that they don't get a good response to oral vaccines," he said. However, it's unclear if this actually happens, because nobody has studied the number of regulatory T cells in such children, he added. —AvB

the gut. Two approaches presented at the meeting used vitamin A or its derivative retinoic acid (RA) to direct immune responses to the gut in mice.

Swantje Hammerschmidt, a postdoctoral researcher in the group of Reinhold Förster at the Medical School Hannover, reported that when combined with RA, subcutaneous immunization of mice with cholera toxin induced gut homing molecules on activated B and T cells, causing them to migrate to the gut (*J. Clin. Invest.* **121**, 3051, 2011). The mice that had been immunized this way had cholera toxin specific B cells and IgA antibodies in the gut, and had no noticeable fluid influx into their intestine after challenge with cholera toxin, suggesting they were protected from diarrhea.

David Schwartz, a senior scientist at Hackensack University Medical Center in New Jersey, used the RA precursor vitamin A to induce immune responses in the gut when he vaccinated mice with chicken Ovalbumin (Ova) as an immunogen. But in contrast to Hammerschmidt, he vaccinated the mice intradermally, which has previously been shown to induce some immune responses in the gut. He also injected molecules that inhibit vitamin D production in the skin, because vitamin D inhibits the effects of vitamin A, and used hairless mice because the oil mice secrete into their hair contains vitamin D. He also injected the cytokine interleukin (IL)-5 to direct the B cells to make IgA, which is the most important and efficient antibody type in the gut.

Two weeks after the second of two vaccinations that were 20 days apart, Schwartz and colleagues found five- to 10-fold higher levels of Ova-specific IgA antibodies in the gut of mice vaccinated this way, compared with mice that had been vaccinated with Ova alone. The addition of vitamin A, Schwartz said, caused the antigen-presenting cells in the skin to educate B cells to home to the gut.

Next, Schwartz plans to use the strategy to direct immune responses to an HIV vaccine candidate to the gut, first in mice, and if the results are promising, in nonhuman primates.

### Improving the gut flora

Bacterial infections and the gut flora are important factors in the complex syndrome of malnourished children with a poorer response to oral vaccines. Therefore, it's important to understand and improve the gut flora in these children.

One way to improve the gut flora is to add probiotic bacteria, the kind

of bacteria that are found in yogurt and are believed to improve gut health. Until recently there wasn't much solid evidence that probiotic bacteria can improve gut health, but that is changing, said Kim Barrett, a professor of medicine at the University of California in San Diego, who studies the effects of probiotic bacteria on the gut. "Historically there has been a lot of belief and not so much scientific evidence," Barrett said. "That situation is rapidly changing. There is plenty of data out there now looking at beneficial effects of these probiotic strains on the gut."

Shinji Fukuda, a research fellow at the RIKEN Institute in Yokohama, Japan, reported how a certain type of probiotic bacteria called *Bifidobacterium* that can be found in yogurt can protect mice from dying from infection with a certain serotype of *E. coli* (*Nature* **469**, 543, 2011).

Fukuda and colleagues found that the preventive *Bifidobacterium* type had a gene that enabled it to import fructose, which the bacterium can turn into acetate. Acetate activates an anti-inflammatory response in colon cells.

Fukuda said food manufacturers could now use this finding to test if the *Bifidobacterium* types they add to yogurt also have the protective fructose transporter gene.

Barrett also studies how pathogenic and probiotic bacteria affect the gut in mice. Using a mouse model where *Salmonella* infection causes diarrhea, she found that the *Salmonella* caused diarrhea because the gut of the mice failed to absorb sodium and chloride ions from the gut lumen, which prevented water from being absorbed from the gut. These insights could lead to the development of drugs to treat diarrhea by restoring the absorptive transport of ions, Barrett said.

Using a different model of mice that have ulcerative colitis (inflammation of the colon) and diarrhea, Barrett and colleagues also found that feeding the mice daily for two weeks with *Lactobacillus acidophilus* and *Streptococcus thermophilus*, two probiotic bacterial strains found in yogurt, could ameliorate diarrhea and weight loss in the mice. *In vitro* experiments showed that the probiotics reversed the negative effects of pathogenic bacteria or inflammatory cytokines on ion transport functions of the gut and on its function as a barrier from pathogens.

Next, Barrett wants to better understand if these probiotics can also improve the symptoms of the mice infected with *Salmonella*. If they can, then probiotics could be a possible treatment for diarrhea caused by *Salmonella* in humans. ■

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# Vaccine BRIEFS

## US Government Commits More Funding to AIDS

At a World AIDS Day event on Dec. 1 sponsored by Irish rock star Bono's ONE campaign, US President Barack Obama announced new commitments to controlling and preventing HIV/AIDS worldwide.

Before an audience at George Washington University that included Bono, the singer Alicia Keys, and prominent research figures like US National Institute of Allergy and Infectious Diseases' director Anthony Fauci, President Obama pledged that by the end of 2013 the President's Emergency Program for AIDS Relief (PEPFAR) would directly support more than six million people on anti-retroviral (ARV) therapy—two million more people than currently receive treatment through PEPFAR—as well as provide ARVs to more than 1.5 million HIV-infected pregnant women. The US will also support more than 4.7 million adult voluntary medical male circumcisions to prevent HIV infection in Eastern and Southern Africa over the next two years, the President said.

Globally, thanks to all HIV treatment programs, nearly 50% of those eligible for treatment are receiving ARVs. “We are winning this fight,” Obama said. “But the fight is not over, not by a long shot.”

Domestically, the Obama administration has also promised to increase funding by US\$35 million to help cash-strapped state

AIDS Drug Assistance Programs (ADAPs) provide ARVs to 3,000 HIV-infected individuals. Obama did not specify how these initiatives would be funded, but he suggested that continuing to focus on lowering both the purchase price and cost of delivering ARVs would help cover the additional costs.

Obama's remarks came on the heels of a new round of data from the Joint United Nations Programme on HIV/AIDS showing that while the number of new infections held steady from 2009 to 2010, the global incidence has declined by more than 21% since 1997, driven by declines in many sub-Saharan nations. However, HIV incidence in the US and Europe has remained static over the same time period while new cases have surged 23% in Eastern Europe and Central Asia since 2008.

AIDS advocates applauded Obama's announcements, especially given the current economic environment. In November, the Global Fund to Fight AIDS, Tuberculosis and Malaria, which accounts for about a quarter of international financing for HIV/AIDS, announced that it wouldn't be making new grants because it has only secured enough funding to sustain ongoing programs through 2013. —*Regina McEnerly*

## Researchers Characterize Target of Broadly Neutralizing Antibody PG9

Following the isolation of dozens of broadly neutralizing antibodies (bNAbs) from HIV-infected individuals, the focus has shifted to characterizing the epitopes on HIV that are targeted by these antibodies. Most recently, a study led by Peter Kwong, chief of the structural biology section at the Vaccine Research Center at the US National Institute of Allergy and Infectious Diseases, provided an atomic-level description of the epitope on the first and second variable loops known as V1 and V2 that form part of the glycan shield of HIV Envelope that is targeted by the bNAb PG9 (*Nature* 480, 336, 2011).

The V1/V2 portion of gp120 is targeted by a number of the recently isolated bNAbs. However, strategies used to determine the structure of other portions of HIV gp120 were unsuccessful in providing a crystal structure of this important region.

To successfully get the structure of V1/

V2 this time, researchers placed the V1/V2 sequences (amino acid residues 126-196) into two protein scaffolds and then crystallized the scaffolds in complex with PG9. These crystals were then used to study at an atomic level the interactions between PG9 and V1/V2. Researchers found that PG9 grasps a specific glycan at amino acid residue 160, forming an extensive interaction that is critical for PG9 binding. Other interactions with PG9 occur at another glycan at residue 156 or residue 170 in the second scaffold, though these are not necessary for PG9 recognition. Additionally, a strand in the CDR H3 region of PG9 interacts through hydrogen bonding with the most variable strand of amino acids in V1/V2 known as strand C. Together with a minor interaction with strand B and the B-C connecting loop of V1/V2, these interactions complete the PG9 epitope.

This epitope is less complex than those of other antibodies, suggesting it may be easier to design and present an immunogen based on it. However, the glycosylation at residue 160, which is necessary for PG9 binding, is not always present in transmitted founder viruses, developing only through the process of immune selection, according to some studies.

Researchers note that there is now more interest in the V1/V2 region, given that the recent correlates of risk analysis from the RV144 trial in Thailand suggested that antibodies targeting this site were associated with a reduced risk of HIV infection (see *A Bangkok Surprise, IAVI Report*, Sep.-Oct. 2011). The study's authors write that, “it is fascinating that the V1/V2 domain—which functions in evading antibody-mediated neutralization—is itself a site of effective neutralization.” —*Kristen Jill Kresge*

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