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The Bulletin on AIDS Vaccine Research

[SPOTLIGHT]

## Scoping out the Gut

A recent conference in India highlighted how malnutrition and gut health affect the immune system and its response to vaccination

Good nutrition and a healthy intestinal environment are important for a healthy immune system and also affect the immune system's response to vaccination. This is particularly important for vaccine responses among children in developing countries.

Data have shown the response rates to oral vaccines for rotavirus—the leading cause of severe diarrhea in infants and children and a lethal scourge in poor countries—to be weaker among children from developing countries, though the response rates to vaccines that are administered systemically by injection—such as the measles vaccine—are much less impaired.

Exactly why children in poor countries seem to have weaker response rates to oral vaccines is not clear, noted researchers attending the Keystone Symposium on Malnutrition, Gut-Microbial Interactions and Mucosal Immunity to Vaccines, held Nov. 7-11 in New Delhi, India, a country where 42% of children under age five suffer from malnutrition, according to a 2012 report from the Naandi Foundation, an independent charitable group. In addition to poor nutrition, many children in developing countries have an unhealthy gut environment, which also contributes to their poor response to oral vaccines. According to 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, malnutrition and immune system dysfunction also likely exacerbate each other, with unfortunate results. Malnutrition seems to cause the gut to fail to serve as a barrier to pathogens, and infection with these pathogens further damages the gut and affects the absorption of nutrients, which in turn makes the malnutrition worse, she said.

However, understanding precisely why vaccines don't work in this environment isn't easy, according to Chris Wilson, director of discovery at the Bill & Melinda Gates Foundation, one of the sponsors of the symposium. The Keystone symposium focused on the role nutrition plays in gut immunity because a better understanding of this role might lead to the development of vaccines that work better in children in developing countries.

Unfortunately, combing the gut for clues is difficult because this process typically requires taking tissue biopsies, an invasive procedure that for obvious reasons is not advisable or done in children. Instead, Kang said researchers have had to resort to indirect methods such as measuring serum or fecal levels of the antibody immunoglobulin (Ig) A, which is produced at mucosal surfaces, such as those lining the gut,



among other places. But just using markers like this antibody isn't ideal, leading the Gates Foundation to announce US\$9 million in grants at the meeting to support research to find better non-invasive markers for assessing gut function and health.

Despite the challenges in determining gut health, researchers are beginning to connect the dots about why malnourished children have a poorer response to vaccination. William Petri, a professor of medicine at the University of Virginia, and colleagues looked at a group of three-year-old children from Dhaka, Bangladesh, and found that the children with more stunted growth were less likely to respond to the oral polio vaccine (OPV), suggesting a possible role of malnutrition.

Evan Newell, a research associate in

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A clearer understanding of how nutrition and gut health impact immune responses in the gut is a potential roadway toward more effective vaccines for children in the developing world, such as those shown above from a village in India. Photo by Andreas von Bubnoff.

Mark Davis's lab at Stanford University, and colleagues also found evidence of chronic inflammation in a subgroup of children in Petri's study who failed to respond to OPV. Chronic inflammation could dampen the response to vaccination because it can ultimately push the immune system to get so burned out that it becomes desensitized to the vaccine. Newell and colleagues actually found evidence that the immune systems of these children are becoming desensitized to the vaccine.

### Improving the gut environment

Given that the gut environment plays an important role in the response to vaccination, researchers are trying to understand how to improve the gut environments in

these children. One way to do this is to add probiotic or "good" bacteria, such as the kinds found in yogurt, which are believed to improve gut health. Until recently there wasn't much solid evidence that probiotic bacteria improve gut health, but that is changing, said Kim Barrett, a professor of medicine at the University of California in San Diego. "Historically there has been a lot of belief and not so much scientific evidence," Barrett said. "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 at the conference that a certain type of probiotic bacterium called *Bifidobacterium* that is found in yogurt could

protect mice from dying from infection with a certain serotype of the bacterium *E. coli*. Fukuda and colleagues found that the probiotic bacteria had a gene that enabled them to import a certain type of sugar. This eventually results in activation of an anti-inflammatory response in colon cells.

In another study, Barrett fed mice that had inflamed colons and diarrhea two other probiotic bacteria strains (*Lactobacillus acidophilus* and *Streptococcus thermophilus*) daily for two weeks. They found these probiotic bacteria strains found in yogurt could ameliorate diarrhea and weight loss in the mice. Next, Barrett wants to better understand if these probiotics can also improve the symptoms of mice infected with the food-borne bacterium *Salmonella*. If they can, then probiotics might be a possible treatment for diarrhea caused by *Salmonella* infection in humans.

### Directing immune responses to the gut

In addition to finding ways to improve the gut environment, researchers are also searching for different ways to deliver orally administered vaccines designed to combat intestinal diseases like rotavirus. Conventional intramuscular or subcutaneous immunizations often only induce weak immune responses in the gut, and therefore protect only weakly against infections that occur there. However, researchers are starting to modify injected vaccines so that they can induce immune responses in the gut. Some approaches involve using vitamin A or its derivative retinoic acid to direct immune responses to the guts of mice. ■

*This Spotlight was adapted by Regina McEnery from an article by senior science writer Andreas von Bubnoff that appeared in the November/December issue of IAVI Report.*

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VAX is a monthly bulletin from IAVI Report, the publication on AIDS vaccine research published by the International AIDS Vaccine Initiative (IAVI). It is currently available in English, Spanish, and Portuguese as a downloadable PDF file or an e-mail bulletin.

The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996, IAVI works with partners in 25 countries to research, design and develop AIDS vaccine candidates. In addition, IAVI conducts policy analyses and serves as an advocate for the AIDS vaccine field. For more information, see [www.iavi.org](http://www.iavi.org).

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## Two New HIV Vaccine Trials Launched in Recent Weeks

Two preventive Phase I AIDS vaccine trials were launched recently, testing two different DNA-based AIDS vaccine candidates. In one trial that began in December, investigators from the UK began enrolling 36 women ages 18-45 at low risk of HIV infection in a randomized controlled trial comparing the safety and immunogenicity of a DNA-based vaccine candidate containing fragments of HIV's spiky outer-surface protein that were isolated from a clade C virus, the most dominant strain circulating in sub-Saharan Africa and the one responsible for infecting half of the world's 34 million people living with HIV.

The trial, known as MUCOVAC2, will be examining three different routes of vaccination. The first group of twenty women will receive a high or low dose of the candidate by intramuscular injection, administered along with the adjuvant glucopyranosyl lipid adjuvant (GLA), which was developed by the Seattle-based non-profit Infectious Disease Research Institute (IDRI), a product-development partnership that is working to develop new technologies that target diseases in developing countries. GLA appears to have the ability to boost both antibody and cellular immune responses.

Another six women will receive the vaccine candidate intranasally in the form of drops, administered together with the adjuvant chitosan, which is derived from the outer skeleton of shellfish and insects and has been found to improve the immunogenicity of other vaccines that are administered mucosally.

Another group of 10 women will receive an intramuscular injection of the vaccine candidate in conjunction with vaginal application of the candidate formulated into a gel. The vaginal gel will be applied nine times in a one-month cycle. This gel version of the vaccine candidate, which has been tested previously in clinical trials on its own, does not contain an adjuvant. Catherine Cosgrove, honorary consultant in infectious disease and general medicine at St. George's University of London, who is leading the study, says the

combination of an intramuscular injection with vaginal gel application aims to induce a more focused mucosal immune response.

"This is the first time the [candidate] is being used intranasally or intramuscularly," adds Cosgrove. Studies in mice, rabbits, and rhesus macaques showed the vaccine candidate was safe and immunogenic. A consortium that includes St. George's, Imperial College, Hull York Medical School, the Medical Research Council Clinical Trials Unit, and IDRI contributed to developing the vaccine candidate. The trial is being funded by the Wellcome Trust.

In another Phase I trial that began enrollment in December, investigators will evaluate the safety and immune responses induced by a DNA-based HIV vaccine candidate, developed by Profectus BioSciences, in a prime-boost regimen. The DNA candidate encodes multiple HIV proteins and is being co-delivered with the adjuvant interleukin-12 (IL-12)—a protein secreted by immune cells in response to viruses or bacteria—to help boost the immune response. The DNA candidate is being followed by vaccination with a viral vector-based candidate that uses an inactivated strain of the cold virus (adenovirus serotype 35; Ad35) to deliver HIV fragments.

Investigators plan to recruit 75 volunteers ages 18-50 from Rwanda, Kenya, and Uganda in this study, known as B004. The trial, which is being sponsored by IAVI, employs a novel technique called electroporation to deliver the DNA vaccine candidate (see box, right). The goal of electroporation, which delivers the vaccine candidate intramuscularly through a series of electric pulses, is to get more of the vaccine into cells.

Enrollment in B004 began in Rwanda in December, with vaccinations slated to begin in Kenya and Uganda in early 2012, pending regulatory approvals. By using Ad35, researchers are hoping to circumvent issues with pre-existing immunity to the viral vector. In the STEP trial, which showed that an Ad5-based vaccine candidate failed to prevent transmission or slow

disease progression in vaccinated volunteers, data suggested that male volunteers who received the vaccine had a higher risk of acquiring HIV if they were uncircumcised and had pre-existing antibodies against the Ad5 vector (see VAX Oct.-Nov. 2007 *Spotlight* article *A Step Back*). Ad35 is less prevalent worldwide than Ad5, and therefore there should be less pre-existing immunity to the vector.

### It's Electric!

#### What is electroporation?

Electroporation (EP) is a vaccine delivery technique that induces temporary pores in the membranes of muscle or skin cells so that these cells can take up the vaccine more easily. Vaccine candidates are delivered using a small hand-held EP device that uses a needle to inject the vaccine and four thin wires to administer electrical pulses that are milliseconds in length.

#### How is it useful in vaccination?

EP has been shown to enhance immune responses induced by DNA-based vaccine candidates in clinical trials.

#### Why is electroporation used for DNA vaccines?

DNA-based vaccine candidates, while safe and effective, are weakly immunogenic due to poor DNA uptake into cells. EP is one way of improving the efficiency of delivery.

#### What are the risks?

The same EP device being tested in the B004 trial was tested in a Phase I trial involving 40 volunteers in the US and found to be safe and well tolerated. Possible adverse events that may occur following EP include a brief twitching of muscles at the vaccination site and a tingling sensation in the arm and fingers for up to one hour after injection. Mild or moderate muscle soreness, redness, bruising, and pain can also occur one to three days afterward. More severe reactions such as dizziness and fainting are rare.

#### Is electroporation a new technique?

EP was discovered in 1970 and has been used in human studies since the early 1990s. Along with preventive vaccines, EP is also being studied in conjunction with therapeutic vaccines and gene therapy. —RM

# Understanding How Viral Vector-based AIDS Vaccine Candidates are Manufactured

What are some of the challenges in developing viral vector-based vaccine candidates and how are scientists trying to overcome those challenges?

By Regina McEnerly

Many of the current AIDS vaccine candidates that are being tested in clinical trials utilize viral vectors to shuttle HIV fragments into the body that can stimulate an immune response (see *VAX* Sep. 2004 *Primer on Understanding Viral Vectors*). Some of the viral vector-based candidates are being tested in prime-boost combinations with other approaches (see *Global News*, this issue). Several different viruses have been used to develop vectors for HIV vaccine candidates, including adenovirus, a common cold virus, and pox viruses, such as modified vaccinia Ankara virus and canarypox, among others.

The viruses used as viral vectors are attenuated so that they cannot cause disease. They are also manipulated, so that in addition to containing their own genes they can carry HIV genes, referred to as antigens, which cannot cause an HIV infection.

The HIV genes that are placed into the viral vector are referred to as the vaccine insert. Once the vaccine candidate is injected into the body, HIV's genetic material is taken up by cells and converted into protein that hopefully will trigger the immune system to respond to HIV. This sounds simple enough, but viral vector-based vaccine candidates present some unique design and manufacturing challenges. Because the manufacturing process is so complicated, additional effort is required to avoid delays in the clinical testing of viral vector-based vaccine candidates.

## Not harmonious

To develop viral vector-based candidates, scientists first need to design and generate the vector with the HIV insert in cells that can support virus growth. The virus vector is then amplified multiple times to produce hundreds of virus particles carrying the HIV genes, which are then subjected to extensive testing.

One of the major challenges in making viral vector-based vaccine candidates

comes down to chemistry, or rather, a lack of chemistry between the vector and the insert. Sometimes the vector and the insert are simply incompatible. For instance, if the length of the insert is too long or its configuration is not suitable to the viral vector, the vector may simply reject the insert. In other cases, the virus acting as the vector may introduce mutations into the HIV genes that may prevent produc-

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**Because the manufacturing process is so complicated, additional effort is required to avoid delays in the clinical testing of viral vector-based vaccine candidates.**

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tion of the complete protein once inside the body. These changes can ultimately impact the generation of a good immune response following vaccination. Sometimes the vector can even clip the insert, rendering it completely useless.

In some cases the vector will tolerate the insert for a while, at other times it will reject it outright. In either case it represents a setback in the production of the vaccine candidate. It is therefore important to analyze the stability of these vectors during the early stages of vaccine development. This is accomplished by subjecting these vector particles to a series of stress tests that assess whether they are stable enough to be tested in a clinical trial. The stress test evaluates the ability of these vectors to express the HIV proteins in cells and in small animals. Even after this vetting process is complete, the vector may still reject the inserts, so it is not uncommon for researchers to have to repeat this cycle several times prior to obtaining a stable vector that

expresses the HIV protein and can be advanced into clinical trials.

## Mosaic antigens

Most of the inserts used in current viral vector vaccine candidates contain HIV gene sequences from a single virus found in a certain region of the world, or a consensus sequence generated from different viruses that are circulating in that region of the world. Because HIV replicates so rapidly, there is tremendous variation among viruses circulating in a population, and even within a single infected individual. To address this, scientists are also attempting to design HIV inserts known as mosaic antigens that are designed to address the overwhelming diversity of HIV (see *VAX* March 2009 *Primer on Understanding How Inserts for Vaccine Candidates are Designed*). Mosaic antigens are based on optimized genetic sequences of the many circulating strains of HIV globally and are meant to induce immune responses to a broad range of circulating strains.

To make mosaic antigens, scientists must stitch together different genetic sequences from multiple strains. These gene sequences do not exist in nature but rather are computer-generated. This makes designing and developing novel viral vector-based candidates that can carry these mosaic antigens even more challenging. Before advancing them into clinical trials, researchers will have to ensure that viral vectors carrying mosaic antigens can be obtained and manufactured at large scale, while maintaining their stability. Researchers are hoping to begin clinical trials with these vaccine candidates containing mosaic antigens soon. ■

