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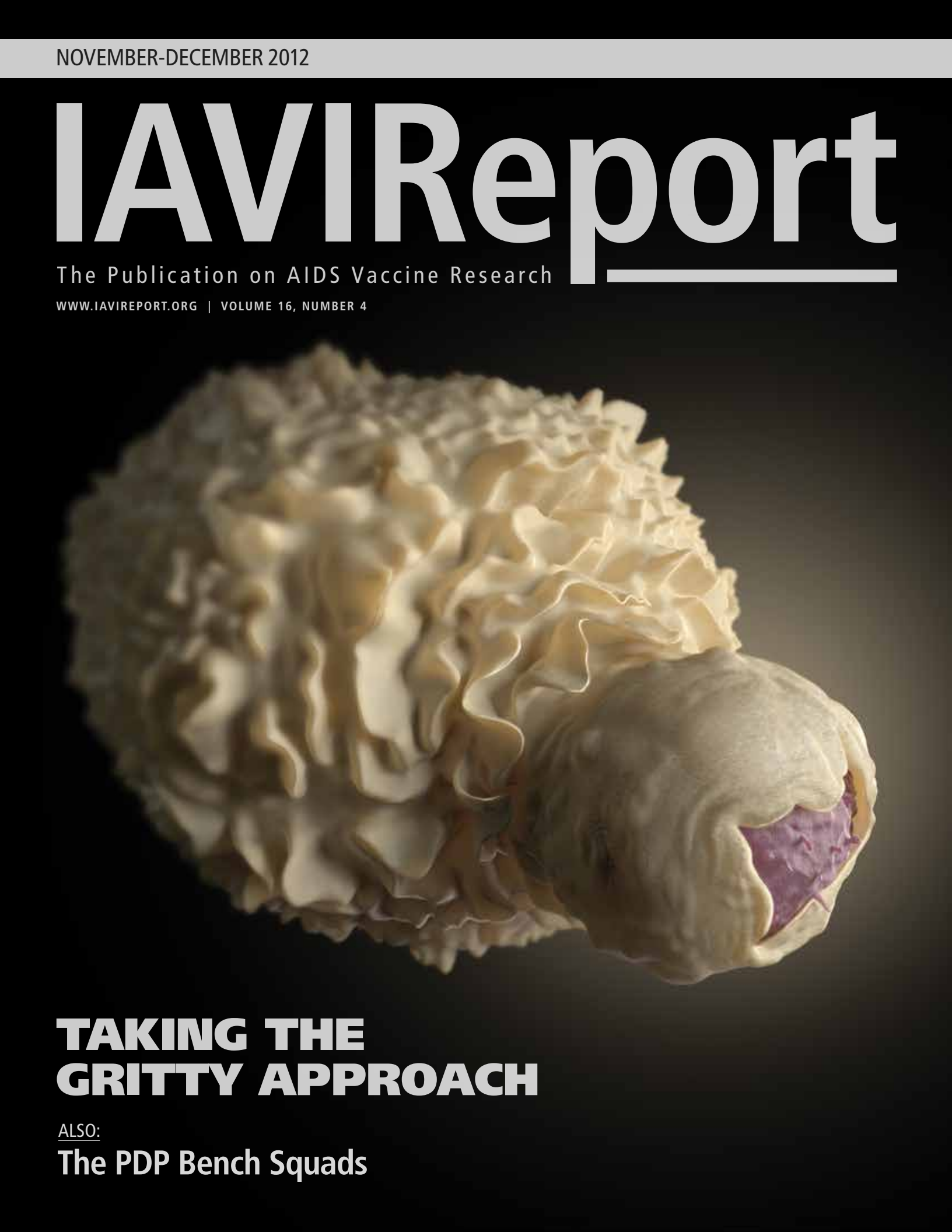


TAKING THE GRITTY APPROACH

ALSO:

The PDP Bench Squads

EDITOR'S LETTER

The editor is well aware that his letters are mere distractions from the brainy feast offered up in the pages of *IAVI Report*. Or maybe something like a menu. So, with no further ado, allow me to introduce this last issue of 2012 to you.

Our reporters ambled down a couple of less trodden paths this winter and returned with engaging stories on matters of relevance not only to HIV research, but to vaccines and global health in general. For starters, Regina McEnery reports on the laboratories that product development partnerships have set up over the years to better address the scientific and technical deficiencies of their chosen fields. How have such ventures panned out? Have they been worth the expense? How are they coping with the current climate of relentless fiscal and economic uncertainty? Read on to find out. (Yes, IAVI's own laboratories are covered in the report. And no, they were given no special treatment, in any sense of the phrase.)

Andreas von Bubnoff, meanwhile, attended a rather specialized conference on particles used to display immunogens, the primary active ingredients of vaccines. Given the impressive track record of particle-based approaches—and the snazzy science behind some of them—we were surprised it took researchers this long to convene a scientific meeting about them. But they finally did, and Andreas took a detour to France to tell us all about it. Enjoy.

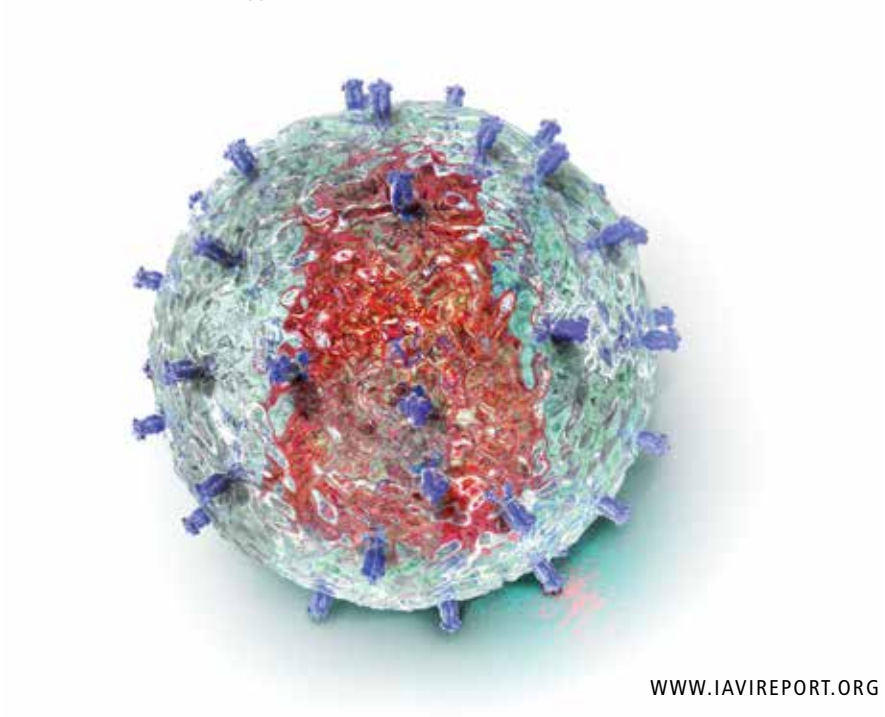
For those more inclined to haikus, we have a few quick reads on the refinements in humanized mouse models that are making them more useful to HIV vaccine research. We also have a short report on a fascinating study on *Staphylococcus aureus* and its interest in a cell-surface receptor favored by HIV, a bit about some changes in leadership in the HIV field, and an article on the somewhat disappointing interim results of the first malaria vaccine candidate to have reached efficacy trials.

As always, we hope you'll enjoy this issue as much as we enjoyed working on it. Happy New Year!

– UNMESH KHER

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. IAVI supports a comprehensive approach to addressing HIV and AIDS that balances the expansion and strengthening of existing HIV-prevention and treatment programs with targeted investments in the design and development of new tools to prevent HIV. IAVI is dedicated to ensuring that a future AIDS vaccine will be available and accessible to all who need it. IAVI relies on the generous donations from governments, private individuals, corporations and foundations to carry out its mission. For more information, see www.iavi.org.

IN THIS ISSUE



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[ON THE COVER]

Artistic visualization of the manner in which a dendritic cell (left; beige) harboring HIV may encase an uninfected $CD4^+$ T-cell (purple) in the context of cell-cell HIV transmission. The rendering is based on 3D electron microscopic imaging of the contact between $CD4^+$ T-cells and dendritic cells incubated with HIV.

Credit: Donald Bliss and Sriram Subramaniam, National Cancer Institute, NIH, Bethesda, MD.

Taking the *GRITTY APPROACH*

Despite their commendable track record and promising future, particle-based recombinant vaccines have never been the sole focus of a scientific meeting. That just changed.

By Andreas von Bubnoff

Vaccines have long been made from live-attenuated or killed forms of targeted pathogens. And the approach has worked rather well: the smallpox vaccine, for example, was made this way and used to end a terrifying disease. But, for safety and other reasons, vaccine developers today tend to favor recombinant vaccines, in which parts of the pathogen are synthesized from scratch and used as immunogens. The resulting vaccines contain either soluble proteins or entire virus-like particles (VLPs), which are similar in size to viruses.

Between the two, VLP-based vaccines have by far the better track-record. Indeed, as Martin Friede of the World Health Organization (WHO) pointed out at a recent conference on virus-like particle & nanoparticle vaccines, both of the approved recombinant vaccines—against hepatitis B (approved in 1983) and HPV (approved in 2006)—are based on VLPs. What's more, another VLP-based vaccine, GlaxoSmithKline's malaria vaccine candidate RTS,S, might well be approved in the near future. By contrast, no non-VLP recombinant vaccine has yet been approved for general use, Friede said.

Not, however, for want of effort. In fact, Friede noted, the bulk of research on recombinant vaccines has focused on non-VLP-based approaches. Between 1983 and 2006, 1284 of the 1339 published clinical trial reports studied non-VLP vaccines. Only 55 evaluated VLP vaccines. In fact, this meeting—held Nov. 28-30 in Cannes, France—was the first solely focused on particle-based recombinant vaccines, according to meeting organizer John Herriot and Gregory Glenn, Chief Medical Officer of Novavax, a company that develops VLP-based flu vaccines, who was on the scientific advisory panel for the conference.

It rained heavily throughout the conference, but there was little reason to go outside: the roughly 140 attendees were treated to talks on particle-based vaccines addressing everything from flu and HIV to the obscure afflictions of salmon.

Particulate matters

Many VLP vaccine candidates are built from viruses that infect bacteria (bacteriophages), or those that infect plants, animals, or even humans. Given the abundance of alternatives, Friede said, there's little reason to use human viruses. Preexisting immune responses to those viruses can, for example, affect immune responses to any vaccines based on them.

That's unlikely to be an issue for vaccines made using synthetic particles. Such vaccines can also have better safety and purity profiles, as they can be made without resort to biological processes.

But what accounts for the greater immunogenicity of particle-based vaccines? In the opening talk of the conference, Martin Bachmann of the University of Zurich said one important consideration is size. The particles can't be too large, as only particles smaller than 200 nm can directly enter lymphatic vessels from vaccination sites, so that they can be transported to the lymph nodes where they activate B cells.

Another important feature is what Bachmann calls "repetitiveness." That is, the particles display the antigen many times on their surface. This is important for a good antibody response, he said, because it enables several B cell receptors (BCRs) to bind an antigen at the same time, which boosts the activation of B cells. Repetitiveness is also important for activation of the complement sys-

tem, which in turn increases B cell activation, he said. As it happens, both of these features—a diameter less than 200 nm, and repetitiveness—are also typical of viruses (HIV is about 100-150 nm across, for example). Further, Bachmann noted, some particle-based vaccines also carry toll like receptor (TLR) ligands on their surface, which activate the innate immune response and so improve both humoral and cellular responses.

VLPs against HIV

VLPs seem to be quite popular with AIDS vaccine researchers. Richard Compans from Emory University presented work on one VLP-HIV vaccine concept to induce antibodies to the membrane proximal external region (MPER) of gp41, which is important for fusion of the viral membrane with the target cell membrane. The VLPs consist of a sphere of plasma membrane that contains the HIV core protein inside and HIV Env on the surface. The recombinant HIV genes are expressed in baculovirus, which infects cultured insect cells, and the VLPs are purified from infected cells.

The researchers boosted the number of Env spikes on the VLPs to ten times the number found on HIV, which typically displays about 10-15 of them. The antibodies induced by these VLPs were not very potent, but became more so when VLPs were also engineered to carry a membrane-anchored form of bacterial flagellin, which activates TLR5 (*MBio* **2**, e00328-10, 2011).

In another study, the researchers made the gp41 part of Env more accessible to the immune system by replacing the gp120 part of the Env spikes with the smaller hemagglutinin (HA1) subunit of the flu virus. Immunization of guinea pigs with the resulting "HA gp41 VLPs" induced antibodies that were specific to the gp41 part of Env and that neutralized tier 1 and 2 viruses (*PLoS One* **6**, e14813, 2011).

One advantage of the approach, Compans said, is that gp41 is presented in its native conformation on the VLP. Compans also described an improved way to deliver the VLP vaccine: using a device with several microneedles, each a fraction of a millimeter long and coated with a VLP-based flu vaccine displaying the HA protein. In mice, this induced 10-fold higher immune responses than did an intramuscular injection with the same amount of vaccine.

Tsafrir Mor from Arizona State University also presented a VLP-based approach to make an HIV vaccine candidate that elicits a gp41-specific antibody response. In this case, the VLPs were made in tobacco plants. Aside from being relatively inexpensive, Mor said, the approach ensures that prions and other contaminants that can be introduced by animal cell lines are not a concern.

Like Compans, Mor and colleagues managed to increase the number of Env spikes on the surface of their VLPs about 10-fold compared to HIV. To expose gp41, they removed the outer gp120 portion of Env. The remaining gp41 retained its transmembrane section, Mor said, to better recapitulate the protein's native shape, especially in regions close to the plasma membrane.

It seems to have worked. Mor and colleagues found that their VLPs, which only contained HIV gp41 and Gag, bound two broadly neutralizing antibodies (bNAbs), 2F5 and 4E10, that target the MPER of gp41. In addition, intranasal immunizations of mice elicited antibodies to the gp41 MPER and to Gag in the serum and in mucosal tissues such as the vaginal mucosa.

Fabien Pitoiset, a graduate student from the Université Pierre et Marie Curie in Paris, reported on a VLP HIV vaccine candidate based on murine leukemia virus (MLV), a retrovirus that infects mice. While the VLPs still contain MLV Gag proteins, the researchers replaced the MLV Env on their surface with HIV Env gp140, which includes the entire part of the HIV Env spike that lies outside the plasma membrane. To increase the number of HIV Env spikes on the VLP surface, they replaced the transmembrane part of gp41 with a transmembrane domain from vesicular stomatitis virus. They produced their "retroVLPs" in a mammalian cell line called 293.

The retroVLPs activated cultured dendritic cells (DCs), and subcutaneous injection into mice resulted in uptake of the VLPs into DCs in lymph nodes and the activation of these DCs. Pitoiset said this resulted in "good" cellular and humoral immune responses to HIV Env, which were further improved if the VLPs carried single stranded RNA inside, which is known to activate innate immune responses through TLR7 and 8.

Pitoiset and colleagues plan to include other TLR ligands such as bacterial flagellin in their VLPs, and to insert conserved parts of internal HIV proteins such as Gag, Nef and Tat into the MLV Gag proteins of their VLPs to improve cellular immune responses.

One challenge for HIV vaccine development is that it takes many years for HIV-infected people to develop the kinds of bNAbs that have been shown to neutralize a wide range of HIV strains. Researchers are currently trying to design vaccines to induce similar antibodies. Such antibodies are highly affin-

ity-matured: their potency and breadth of neutralization derives from extensive hypermutation, which makes them very different from their germ line precursors. This means that people injected with vaccines devised to elicit bNAbs may not be able to produce the desired antibodies until much later—perhaps even a few years after vaccination. Of course, this would be very far from ideal.

One way to begin to address this problem is to develop a vaccine that contains proteins or peptides that preferentially bind the germ line precursors of HIV-specific bNAbs (see *Vaccines to Antibodies: Grow Up! IAVI Report*, July-Aug. 2010). At the meeting, John O'Rourke, a research assistant professor in Bryce Chackerian's lab at the University of New Mexico in Albuquerque, presented initial steps toward the development of such a vaccine.

In collaboration with Gary Nabel's lab at the National Institute of Allergy and Infectious Diseases' Vaccine Research Center, O'Rourke performed a VLP-based screen to find peptides specific to the germ line precursors of bNAbs that target the CD4 binding site: VRC01, VRC04 and CHAVI31. The researchers made a library of VLPs, where every VLP presents one of billions of different random peptides on its surface.

To make the VLPs, they used bacteriophage protein MS2 which, when expressed in the bacterium *Escherichia coli*, self-assembles into a 90-subunit VLP, and inserted billions of random 6-10 amino acid peptides into the MS2 protein. They then screened this library for VLPs that best bound the germ line versions of the bNAbs.

O'Rourke said their approach resulted in the generation of VLPs that each contained the RNA molecule that encoded the antibody-binding peptide presented on its surface. This clever arrangement allowed researchers to quickly identify the sequence of the peptides that were displayed on the VLPs that bound the germ line antibodies most strongly.

Using this system, O'Rourke's lab has identified several VLP-associated peptides that bind strongly to the germ line precursors of all three bNAbs. Nabel's lab has shown that one VLP that binds to the CHAVI31 germ line precursor, and two that bind to the VRC04 germ line precursor, can also activate BCR signaling *in vitro*.

O'Rourke said immunization experiments of humanized mice are underway to identify the kinds of immune responses these VLPs generate. The ultimate goal, he said, is to develop a vaccination regimen that can kick start the affinity maturation process and guide it towards the production of bNAbs. This could be accomplished, he thinks, by priming with a germ line binding vaccine, and boosting with a vaccine carrying antigens that are more similar to the actual HIV components.

Toward broader flu vaccines

Flu was a focus of the meeting as well. Peter Pushko, president and CSO of the company Medigen, presented an approach that, he said, for the first time makes it possible to present proteins from three different flu strains on the surface of one VLP. This could help reduce costs, Pushko said, because it enables researchers to manufacture just one particle against three different strains of seasonal or pandemic influenza. He said production of the VLPs, from generating antigens with the right sequence to the final VLP, only takes 6-7 weeks, which would make it easier to quickly respond to new flu strains.

To make such trivalent VLPs, the researchers expressed several flu proteins in baculovirus-infected insect cells: Hemagglutinin from three different flu strains, neuraminidase, and the matrix protein M1. Pushko showed that such VLPs containing HA from three different pandemic viruses were immunogenic in ferrets, which are a well-established animal model in flu vaccine development. They also protected ferrets from challenge with each of the three corresponding pandemic strains. The same was the case with VLPs that contained HA subtypes derived from three different seasonal influenza viruses (*Vaccine* **29**, 5911, 2011).

Synthetic approaches

Many vaccine candidates described at the conference were made in cell lines, plants or bacteria. But synthetic particle vaccines are no less intriguing. Arin Ghasparian, CEO of the Zurich-based company Virometix, reported that his company has been developing synthetic VLPs that are 20-30 nm in size and contain 72 monomers. Each monomer has a lipid portion and a protein portion. The protein portion has the shape of a "coiled coil," which can bind to the coiled coil portion of other monomers. Make a mix of the monomers, and the VLPs assemble themselves into particles with peptide exteriors and lipid-lined interiors. Immunogens that are to be displayed on the surface of the VLP are attached to the protein part of the monomer before VLP self-assembly takes place.

Ghasparian said one advantage of this system is that it takes only a week to make a desired monomer. Another is that the resulting VLPs are uniform, well defined, easy to purify, and stable outside the fridge. Ghasparian presented preliminary results obtained using a Virometix VLP

that displays a 24 amino acid part of the HIV Env V3 loop on its surface. This stretch of V3 has previously been shown to bind to F425-B4e8, one of the few V3-specific mAbs that can neutralize HIV strains from different subtypes. In addition, the structure of this V3-antibody pair has been determined (*J. Mol. Biol.* **375**, 969, 2008).

Ghasparian and colleagues grafted onto their VLPs a version of this V3 peptide that had been stabilized in the conformation recognized by the F425-B4e8 antibody. They found that immunization of rabbits with these VLPs induced V3-loop-specific antibodies that could neutralize the same HIV strain that had served as the source of the V3. It also induced antibodies that neutralized other HIV strains, but only after removal of the HIV Env V1 and V2 loops, which in the intact Env spike occlude the V3 loop (*Chemobiochem.* **12**, 2829, 2011). This means that the V3-VLPs are not useful for development into an HIV vaccine candidate. But it shows that, in principle, this VLP system can be used to develop vaccines that elicit functional immune responses *in vivo*, Ghasparian said.

Ghasparian and colleagues also immunized rabbits with VLPs that contain the malaria circumsporozoite antigen that is also used in the RTS,S vaccine candidate. They found that this induced antibodies that bound to whole malaria sporozoites.

Julianna Lisziewicz, president and CEO of the company Genetic Immunity, presented a therapeutic vaccine approach that's based on synthetic nanoparticles and intended to induce an immune response that kills infected cells in HIV-infected individuals. The immunotherapy, as Lisziewicz calls it, is named DermaVir and consists of synthetic nanoparticles with diameters between 70 nm to 300 nm. Each carries a single plasmid DNA inside that encodes all 15 HIV proteins, two of which are nonfunctional to ensure that the resulting HIV particles cannot integrate or replicate (*Vaccine* **29**, 744, 2011). This, Lisziewicz said, makes DermaVir the most complete vaccine modality in terms of the number of HIV epitopes used.

Before DermaVir is used, a rough sponge is rubbed over the application site to disrupt the outermost epidermal layer (stratum corneum). Then the skin area is covered for three hours with about 10^{13} nanoparticles, which are applied as a liquid and contained by a patch. To improve the immune response, the patches are applied at different locations, so that responses are simultaneously activated inside a number of different lymph nodes.

Lisziewicz said data from mice, rabbits and monkeys show that some of the particles are taken up by Langerhans cells, the antigen presenting cells (APCs) of the skin. They then carry the particles with them as they migrate to lymph nodes, mature into DCs and express the HIV genes from the nanoparticles. The resulting HIV VLPs contain all HIV proteins but cannot integrate into the genome or replicate.

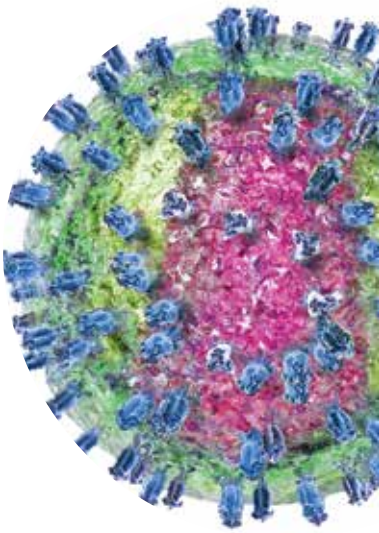
Evidence from mice, monkeys, and human trials shows that these HIV VLPs can induce HIV-specific central memory $CD8^+$ T-cell responses with broad epitope specificity and "high proliferative capacity," Lisziewicz said. This, she added, is the same type of immune response that keeps HIV replication under control in long term nonprogressors (*J. Virol.* **86**, 6959, 2012). A recently completed phase II trial called GIEU006, she said, showed that treating HIV-infected patients, who were not on highly active antiretroviral therapy (HAART), with DermaVir four times in 24 weeks resulted in a 70% reduction of viral load, compared with placebo-treated patients. To Lisziewicz, this suggests that the $CD8^+$ T-cell response induced by DermaVir kills HIV-infected cells. The effect of DermaVir on viral load is, however, less potent than HAART. Lisziewicz hopes that this can be improved by customizing the HIV sequences in the vaccine DNA so that as many epitopes as possible overlap between the vaccine and the HIV strains the patient is infected with (and the parts of these HIV strains that are presented to the immune system).

A recent phase I trial showed that DermaVir also induces an HIV-specific CD8+ central memory T-cell response in people on HAART (*PLoS One* **7**, e35416, 2012). Next, Lisziewicz plans a clinical trial to study if such people continue to show undetectable viral loads after they interrupt treatment. "We are hoping to show that at least some of these patients will respond," and retain undetectable viral loads after treatment interruption, she said, adding that such patients could then be given DermaVir once a year to keep the virus suppressed.

One advantage of DermaVir treatment is that it has fewer side effects than HAART. And because DermaVir kills HIV infected cells, Lisziewicz hopes that it will reduce the HIV reservoir, setting the stage for the successful application of a future curative therapy.

Synthetic particle vaccines are also being used to bridge a gap in vaccine development: the need for vaccines that protect newborns from many infectious diseases. Ofer Levy of Boston Children's Hospital and Harvard Medical School said that some established vaccines, for example polysac-

Continued on page 19



Seizing THE REINS

From translational science to manufacturing, PDPs have significantly expanded their mandates and built labs that they believe fill an important niche in global health research. Is this a good idea?

By Regina McEnery

Three decades ago, three researchers from Seattle concerned about poor access to birth control options in developing countries put their heads together and created the nonprofit organization known today as PATH. Since then PATH—the current name derives from Program for Appropriate Technology in Health—has far exceeded its initial mandate. It has grown to become a major participant in global health research and a leading force for the development of vaccines of relevance to people in developing countries. A leader or member of many product-development partnerships (PDPs), PATH isn't just a sponsor of other people's ideas. It also has a sophisticated laboratory of its own that has grown steadily since it opened its doors in 1979. Today, the PATH lab has one team working on a variety of vaccine stabilization and formulation problems, and another dedicated to diagnostics.

In the world of PDPs, PATH is far from unique in this regard. Other organizations of its kind, such as the Infectious Disease Research Institute (IDRI), Aeras and IAVI have also built their own sophisticated laboratories to speed the translation of scientific discovery into global health solutions or to address specific problems of vaccine development, from formulation to assay development to heat stability. These labs don't come cheap. Nor are they easy to manage. They are dependent on flexible funding, which isn't always easy to get, and are often difficult to staff due to their specialized functions. On the other hand, they have generated a fair number of technical breakthroughs of potentially great relevance to global health. That is of course what they're supposed to do. What is less clear, though, is just how much of this they should be doing on their own.

PDPs are meant to serve as bridges between the public and private sector, harnessing the expertise of each sector to tackle a problem of relevance to economic development or global health. The idea is that academic and other non-industry researchers—who might have splendid ideas and high ideals but little product development experience—can work through PDPs to tap the resources and expertise of manufacturers. To make that proposition attractive to the private sector, the typical biomedical PDP will take on much of the financial risk of developing products to deal with complex diseases that aren't likely to generate much profit.

PATH's Malaria Vaccine Initiative (MVI), for example, in partnership with GlaxoSmithKline (GSK), is supporting the development and clinical testing of the RTS,S vaccine candidate against the malaria parasite. In 2001, MVI forged an agreement with GSK to develop RTS,S for African infants and children, offering technical assistance and alleviating much of the risk of the venture for the drug company by providing funding through a grant from the Bill & Melinda Gates

Foundation to MVI. A large-scale Phase III efficacy and safety trial has been underway at 11 sites in seven African countries. The latest set of results, published in the *New England Journal of Medicine* in November, suggests that the candidate is only modestly effective in preventing severe and clinical malaria in infants (see *Vaccine Briefs*, this issue). Final results from this trial are anticipated by the end of 2014.

But PATH also does a good deal of its own research, operating a biosafety level-2 (BSL-2) lab at its headquarters in downtown Seattle. A Vaccine Formulation and Stabilization Team at the lab has, with a number of external technical collaborators, formulated a vaccine candidate against enterotoxigenic *E. coli* (ETEC) and a veterinary vaccine against Newcastle disease as fast-dissolving tablets for oral immunization. The tablet technology PATH has advanced could support the creation of inexpensive, heat-stable and easy-to-use vaccine products that can be dissolved in water and swallowed or administered under the tongue—all of which are of significant value to public health in developing countries. Just this summer, PATH's lab in partnership with several other collaborators launched a project to expand global access to adjuvants of special relevance to vaccines made from highly purified antigens, which tend to be less immunogenic than whole-organism varieties.

Indeed, since it opened its doors more than three decades ago, the size and scope of the laboratory has expanded dramatically. While the non-profit's mission extends to nutrition, maternal and child health and improving health systems technology, its in-house staff of vaccine scientists primarily concentrate on solving the technical problems of vaccine development, effectiveness and ease-of-use in low-resource settings. The direct involvement of PATH's scientists in such efforts, the organization argues, has allowed it to monitor and maintain the quality of projects and products with which it is closely associated. According to Debbie Kristensen, who leads the Vaccine Technologies group at PATH, this soup-to-nuts approach is unusual in the PDP world. "We are involved in every aspect of vaccine development and introduction, so we have an understanding of all the pieces of the puzzle."

Other PDPs that operate BSL-2 labs include PATH's neighbor in Seattle, IDRI, which focuses on diagnostics, vaccines, and therapeutics for neglected tropical diseases, such as leishmaniasis and schistosomiasis, as well as AIDS, malaria, and tuberculosis. Aeras, which is based in Rockville, Md., and dedicated to developing better tuberculosis vaccines, has its own laboratories and manufacturing facilities. IAVI, which publishes this independent magazine, has its AIDS Vaccine Design and Development Laboratory (DDL) at the 95-acre Brooklyn Army Terminal, which was built in 1918 and recently converted into a bioscience park.

IDRI's research presence has grown substantially in recent years with the development of a glucopyranosyl lipid adjuvant, or GLA. The novel toll-like receptor 4 (TLR4) agonist has since been added to candidate vaccines for AIDS, influenza, malaria, tuberculosis, visceral leishmaniasis, schistosomiasis, and hookworm. The adjuvant stimulates innate and adaptive immune responses by inducing dendritic cell maturation and the release of pro-inflammatory cytokines and chemokines associated with cell trafficking (*PLoS One* **6**, e16333, 2011).

The DDL, which opened in 2008, turned its attention early to systematically and briskly devising and testing potential HIV immunogens. This is precisely the kind of thing biotech and pharmaceutical companies do to develop novel vaccines and IAVI felt it was largely lacking in the AIDS vaccine field. "The idea for the DDL was to go into that space that is often occupied by biotech and supplement it with capacity to test immunogens rapidly and efficiently," says Rick King, IAVI's vice president of research and development. That work was facilitated by its access to a nearby animal testing facility owned by the State University of New York Downstate Medical Center.

Aeras, meanwhile, has focused on manufacturing, most recently for its own live recombinant tuberculosis vaccine candidate that was evaluated in a Phase I trial by researchers from St. Louis University in Missouri. The vaccine candidate, called Aeras-422, is a modernized version of the bacille Calmette-Guerin (BCG) vaccine, which is derived from an avirulent bovine strain of the TB bacterium and is primarily given to children in the developing world. Aeras-422 was dropped from further development after two of the participants in the 22-person trial developed shingles. But the PDP manufacturing facility, which adheres to good manufacturing practices (GMP) enforced by the US Food and Drug Administration, has kept itself busy on other projects. It is currently engaged in process development, fermentation, protein purification, and fill-and-finish—the filling, sealing, and labeling of bulk vaccine product

in vials—for vaccine candidates against not only TB, but other diseases as well.

This represents a bit of a change from the initial vision for the manufacturing facility. Aeras initially restricted the facility to upstream manufacturing of the rBCG vaccine candidate—mostly for recombination BCG fermentations, which are particularly difficult because the bacterium is so fragile and unstable. Eventually, however, it added a second facility to build its capabilities in downstream manufacturing, including the standard lyophilization or spray-drying of vaccine candidates for longer shelf-life.

PATH's in-house lab, for its part, focuses on improving the formulation of vaccines, which is complicated because vaccines often have large, complex biomolecules as active ingredients. Funded by a US$5.2 million contract awarded in 2010 by the Biomedical Advanced Research and Development Authority (BARDA), a branch of the US Health and Human Services, PATH scientists are busy these days stabilizing influenza vaccines. They are investigating heat-stable formulations for existing inactivated and live-attenuated seasonal and pandemic influenza vaccines. They're also developing freeze-drying, foam-drying, and spray-drying technologies to improve the shelf-life of both types of vaccines.

Ties that bind

There has lately been much cross-fertilization between various PDPs or the nonprofits that created them. PATH and IDRI, for example, teamed up two years ago to see whether IDRI's oil-in-water emulsions boosted immune responses to pandemic flu vaccines. PATH sponsored the research, and IDRI conducted the emulsion analysis, focusing on a model antigen representing the main components of a vaccine candidate against the virulent H5N1 influenza virus. The study, which evaluated the influence of oils, surfactants, and excipients on stability as well as antigen structure and immunogenicity of the influenza antigen, found the adjuvant activity by the different oil-in-water emulsions varied quite a bit. But IDRI scientists attributed the differences to the biological activity of the oil composition rather than physical interactions of the antigen with the emulsion (*Influenza Other Respi. Viruses*, doi: 10.1111/irv.12031, 2012).

Likewise, IAVI and Aeras, which have worked together in the past, announced this summer a "collaboration agreement" that will enable the PDPs to share clinical research center capacity for early phase clinical trials and leverage each organization's expertise in the design development and production of vaccine candidates. IAVI also forged a partnership with PATH's MVI this year to provide interferon-gamma ELISpot and multicolor flow cytometry assays as MVI moves malaria vaccine candidates into clinical trials. The T-cell assays were refined and validated at IAVI's Human Immunology Lab at Imperial College London.

For its TB candidate, IDRI manufactured the adjuvant and collaborated with a group in Iowa to manufacture the antigen, while IDRI and Aeras joined forces to execute the clinical testing for the Phase I trial. Aeras' manufacturing facility, meanwhile, has collaborated with the Sabin Vaccine Institute in Washington, whose PDP on the campus of Baylor College of Medicine in Houston focuses on vaccine candidates for neglected tropical diseases, such as Chagas disease and schistosomiasis.

Aeras

A biotech with a social mission, Aeras was established to find better vaccines to fight tuberculosis. Its vaccine discovery and immunology laboratories opened in 2003. Aeras also established an upstream manufacturing facility in 2006 and a downstream manufacturing facility three years later.

Location of lab: Rockville, Md.

Square footage: 28,858, which includes its labs and two manufacturing facilities

Lab and manufacturing workforce: 55

Post-docs: 13

Graduate students: 12

Photo courtesy of Aeras

Aeras completed a master services agreement with PATH earlier this year, which enables Aeras to contract with each of the various PATH organizations focusing on vaccine development for emerging and epidemic diseases. Aeras and PATH have already engaged on two projects this year and expect to pursue additional projects in the future.

Aeras' facilities have also manufactured the first pilot-scale lot of Sabin's *Na*-GST-1 vaccine candidate that is now being tested in a Phase I study in Brazil. The antigen in the vaccine candidate is the glutathione *S*-transferase (GST) protein found in the human hookworm *Necator americanus,* which is critical for blood feeding and survival of the parasite and therefore represents a good target for vaccination. Sabin's PDP also contracted with Aeras to make a pilot-scale lot for its vaccine candidate against schistosomiasis, which afflicts more than 200 million people around the world. Taken together, hookworm and schistosomiasis represent the most common of the seven major neglected tropical diseases, and the second highest burden of parasitic disease behind malaria.

"From our standpoint, in terms of cost and availability for producing Phase I clinical material, we have found PDPs together with developing country manufacturers work well for us because they have a unique commitment to public health," says Sabin's president Peter Hotez. "We have found the interactions have been easier and there is more give and take." Still, working with other PDP labs has drawbacks. "For one, there's the availability of time—they have their own projects to work on," he says. "The other is experience with new vaccines."

PDP labs and manufacturing facilities have turned out to be a magnet for scientists with highly specialized expertise, such as fermentation technology or vaccine formulation and delivery. They are also in some cases vital to the portfolio management of PDPs, doing the preclinical testing required to make decisions about which vaccine candidates to pursue.

Aeras' lab, for instance, is equipped to do flow cytometry, which it uses to measure antigen-specific cellular immune responses that some TB vaccine candidates are designed to provoke. IAVI's DDL is set up to advance vaccine candidates into clinical trials. It is capable of standardized protein production and purification, which is needed to make protein-based experimental vaccine candidates for preclinical studies. It also supports ongoing research at the dozen or so labs in IAVI's neutralizing antibody consortium (NAC). In keeping with its title, the DDL, and its partners at the Bill & Melinda Gates Foundation have also poured money into a replicating viral vector vaccine program that includes replication-competent canine distemper virus (CDV) and vesicular stomatitis virus (VSV) viral vectors.

While other labs are developing AIDS vaccine candidates using VSV, IAVI's is taking a slightly different tack from most. Its VSV vector strategy seeks to induce the immune system to produce an antibody response to HIV. The CDV viral vector vaccine candidate is not currently being used anywhere else, either commercially or experimentally, making the project particularly innovative. "The idea percolated that the measles virus or CDV would be good vectors to deliver a vaccine specifically to the regions [in the body] where HIV likes to infect cells and replicate," says Chris

Infectious Disease Research Institute

The Infectious Disease Research Institute (IDRI) was founded in 1993 by Steven Reed to apply advances in immunology to the development of novel diagnostics, vaccines, and therapeutic products for diseases that disproportionately affect developing countries, including leishmaniasis, tuberculosis, malaria, leprosy, and AIDS. IDRI occupies several floors of a 1960s-style building in Seattle that once housed the Fred Hutchinson Cancer Research Center.

Location of lab: Seattle

Square footage: 50,000. Space includes biosafety level 1 and 2 labs, a GMP production lab, and a vivarium. The lab will expand to 58,000 square feet when IDRI moves to a new facility in Seattle in 2013.

Lab workforce: 89

Post-docs: 7

Graduate students: 2 beginning in 2013

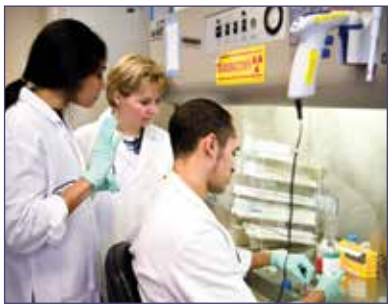


Photo courtesy of IDRI

Parks, IAVI's senior director of viral vaccines and deputy director of the DDL. Parks says both CDV and its cousin, the measles virus, were considered as vectors because they replicate in some of the same tissues as HIV. But the DDL settled on CDV because so many people have pre-existing immunity to measles virus and because other research groups were developing measles virus vectors. "It's not the perfect solution to pre-existing immunity because measles antibodies will cross-react with CDV," says Parks. "But they don't seem to be nearly as potent against the canine virus."

Pre-clinical studies in ferrets, which are susceptible to distemper, have been encouraging. The animals showed no apparent side effects after being vaccinated intranasally or intramuscularly with a CDV vector vaccine candidate that was modified to deliver several proteins from the simian immunodeficiency virus (SIV). They also found that the live vector replicated in lymphoid tissue in the abdominal cavity of the ferrets. "So it was doing exactly what we thought it would do, and the ferrets didn't seem to mind," says Parks.

Importantly, the ferrets also produced antibodies against the SIV proteins showing that the CDV vector replicated enough to induce a response by the immune system. "One of the biggest hurdles we face developing live replicating vectors is balancing safety vs. replication. We need the vector to replicate enough to trigger an immune response, but not so much that it causes symptoms of an infection. In the lab, we say, is it hot enough to make a good immune response without causing adverse reactions?" The lab has grown larger batches of the CDV and VSV vaccine vectors and is now investigating different vaccination regimens in rhesus macaques. If those studies generate promising results, the lab will conduct a challenge study in macaques to see if the vaccine candidate is protective against SIV infection.

As this program illustrates, having its own lab can allow a PDP to invest in promising projects that might otherwise wither on the vine because they present too high a risk of failure. In line with the missions of their parent PDPs, the labs can also help accelerate research on novel vaccines and drugs for diseases that have a disproportionate impact on the developing world—but little commercial appeal to drug companies and biotechs.

Toughing out the rough times

On the flipside, ambitious as they are, these facilities are expensive to staff and maintain. And their financial future is largely tied up with that of the PDP, which itself is dependent upon the largesse of public and private donors. Just how dependent was all too clear when the collapse of the US economy in 2008, and then the Eurozone crisis, forced many wealthy countries—the lifeblood of many PDPs—to scale back or shelve their foreign aid commitments. The recent economic turbulence has also made it much more difficult for PDPs to find new donors and raise additional revenue. IAVI, for instance, was forced to freeze departmental budgets, reduce staff, and curtail programs, including those at its labs. This process has been particularly hard on the DDL, whose staff peaked at about 50 two years ago, but is now down to 26.

"Obviously we are in the midst of a lot of changes, and we need to think about where we're going," says King, in August, just days after IAVI announced a restructuring. King says the DDL

PATH

The Program for Appropriate Technology in Health (PATH) was founded in 1975 with a focus on family planning. Its mission has evolved significantly over time to include other global health causes, such as the development of vaccine candidates and diagnostics for an array of diseases. In its lab, which doubled in size when PATH moved to its current headquarters in 2010, teams are advancing innovative work in diagnostics and vaccine formulation and stabilization.

Location of lab: Seattle

Square footage: 5,465, which is used by the Vaccine Technologies Group's stabilization and formulation team and the Diagnostic Development Group.

Lab workforce: 13

Post-docs: 5

Graduate students: PATH currently does not employ any graduate students

©PATH/Patrick McKern

will curtail the protein production work it has been doing for IAVI's Neutralizing Antibody Center in La Jolla, as well as for other research collaborators. Some of the analytical testing will have to be done in La Jolla as well. "We will have a core mission taking technology from one stage to the next," he says, "but we will have to be more selective about being a very broad hub for field-wide testing of immunogens. We are not going to be able to have quite as open a door and [we'll have to] choose carefully so we fulfill our commitments to partners."

Kristensen says PATH too has felt the effect of the economic slowdown, though this has not led to a drop in the utilization of its laboratory, which was intentionally equipped with standard equipment of relevance to many R&D processes. "While our lab is small in comparison to many, we have actually seen a slight growth in utilization over the last few years," says Kristensen. "During this period, PATH has been fortunate to continue to attract support for our efforts, albeit in slightly different ways than in the past." For instance, though PATH has experienced a decrease in the availability of flexible funding from donors to conduct research on new innovations, this decline has been offset by what Kristensen calls an "increased donor interest in our vaccine formulation, vaccine stabilization and diagnostic assay development capabilities."

IDRI's CEO Stewart Parker says, meanwhile, that because her organization has a history of being extremely frugal the economic downturn has had a relatively minor impact on its programs. "In fact, since 2008, IDRI has increased employment from 73 to 125," she says. "That being says, we'll continue to watch our budget closely, find creative ways to supplement grant income and continue to increase awareness of IDRI's research achievements in order to attract additional unrestricted funding."

One rather ironic upshot of the economic uncertainties faced by PDPs is that their scientists, who often joined the nonprofits in part to escape the daily grind of chasing grants, are now being forced to churn out grant proposals to tap funds from the US National Institutes of Health and other government agencies. Unfortunately, this kind of sponsorship is harder to secure these days, thanks to a larger pool of applicants and static funding.

Nonprofits that invest in their own labs must also contend with the vagaries of biomedical product development and the complexity its inherent unpredictability introduces to portfolio management. And, since doing the right thing—not the profitable thing—is their mission, PDPs often must grapple with how best to share their intellectual property with others and make sure that the tools and products resulting from their research are made available to those who most need them—the world's poorest people. That is, of course, if they can be sure they will have control of the product at that point.

"The typical PDP doesn't have the intellectual property to wheel and deal," says Erik Iverson, IDRI's executive vice president of business development and external affairs. "They really don't have their own products. That is the nature of the PDPs; they are a functional unit created to manage portfolios. So they find organizations that have products in development and enter into co-development agreements to do the work. A few

IAVI Design and Development Lab

IAVI's AIDS Vaccine Design and Development Laboratory (DDL) opened in 2008 at the Brooklyn Army Terminal, which city, state and private entities have been developing as a bioscience center. The DDL has functioned as a hub for IAVI's other laboratories, which include the Human Immunology Lab in London, the Neutralizing Antibody Center in La Jolla, Calif., and the HIV Vaccine Translational Research Laboratory in India. A primary focus of the DDL has been providing translational research support for researchers designing immunogens for AIDS vaccine candidates. It also has an active replicating viral vector vaccine candidate program.

Location of lab: Brooklyn, NY

Square footage: 40,000

Lab workforce: 26

Post-docs: 1

Graduate students: 1

Charlotte Raymond Photography / IAVI

PDPs have their own technology and horses in the race, such as Aeras' recombinant TB vaccine [Aeras 422], but the vast majority of the pipeline belongs to other organizations. That is the nature of the public-private partnership."

Iverson says IDRI is an unusual player in the PDP field in that regard. It creates most of its own products in-house, including vaccine candidates for TB and leishmaniasis, and the organization's bread and butter—oil and emulsion adjuvants. "My point is that we are not middle-men," he says. "We create the stuff pushed forward with funding that we receive. Other PDPs usually go out and find the technology and products."

Necessity and invention

For the longest time, vaccines were made by chemically attenuating or killing pathogens, or finding genetic variants of pathogenic organisms that were naturally attenuated, and delivering them whole to induce immunity. But as standards for both quality and safety have risen in recent decades, researchers have increasingly favored recombinant approaches to vaccine design and manufacturing. Today, those messy or time-consuming techniques have largely given way to more surgical and scientifically sophisticated strategies for vaccine design and delivery. Researchers today use such technologies as viral recombination, codon de-optimization and microRNA insertion to attenuate target organisms, and bioinformatics and protein engineering to devise immunogens. New vehicles for immunogen delivery, meanwhile, include everything from plasmids to replication-competent vectors to bacteria.

All this can complicate vaccine development, and the management of that process. Cutting-edge strategies certainly require staff with rarified skills. But so does the basic business of vaccine formulation and manufacturing, particularly if the targeted pathogen is less than amenable to laboratory cultivation and manipulation. So for all the emphasis on rational vaccine design these days—that is, using antigens, delivery systems and adjuvants that elicit predictable immune responses against specific epitopes—vaccinology remains as much an art as a science, says Pat Fast, a pediatrician and chief of medical affairs at IAVI. "If you could be perfectly rational, there wouldn't be this art to it," says Fast, who has been involved in numerous vaccine trials for AIDS, influenza, and other diseases. "There is a lot of art to getting a virus to grow to high titers, getting a high output of your protein to your cell line. It's not easy and there are not a lot of people who know how to do that."

Just ask Reginald Kidd, Aeras' director of manufacturing and validation. "The current [BCG] vaccine that is on the market is a pretty primitive culture that is grown in not a terribly controlled way," says Kidd. "The modern candidate is a recombinant that expresses proteins from actual mycobacterium TB. The challenge is to wean the bacteria off of the media, bank that culture, to be able to develop a fermentation process that [allows you] to grow and harvest the bacteria but genetically still have the same culture you started off with."

If it sounds difficult, that's because it is. Kidd, who describes himself as an "ex-fermentation guy," came to Aeras several years ago with a background in *Escherichia coli,* a bacterium that multiplies profusely and is thus much easier to deal with, at least from a culturing perspective. "*E. coli* doubles every 20 minutes, BCG may double every 24 hours," says Kidd. "I was amazed at how long it took. It takes a month for all the colonies to show up on the plate. And after all the manipulations you do, the harvesting and washing of the cells, then freeze-drying, it loses its viability. The challenges are growing the bacteria and keeping it alive and having it end up in a vial, freeze-dried, without contaminating it along the way."

Kidd says the lab constructed about a dozen recombinant BCG candidates, before they were able to find one that retained its genetic stability through to the end of the fermentation process. "You get to a point where, maybe two weeks go by, and the DNA inserts are still there. Just to get one passage [subculturing cells] takes a month. So you could construct a [candidate], go through passages, and wait six-to-eight months and say this looks good or take the risk and manufacture right away."

Fast says vaccine PDPs anticipated such difficulties and prepared for them, which explains the establishment of some PDP laboratories. "Some groups have felt that they need to have manufacturing in house, particularly when there is a specialized aspect to growing the protein, like with mycobacterium," she says. Others, she says, have outsourced such work. But the focus a PDP laboratory can provide—at least on products of relevance to its parent organization—has its advantages. "The ideal," says Fast, "is that the lab can do rapid, iterative work without a profit motive, and bring something that is thought to be at the point of being able to be handed over to the commercial sector, even though there may not be a huge amount of profit." ■

Will Humanized Mice Move Us Closer to an AIDS VACCINE?

A recent spate of studies suggest researchers are finding ways around the limitations of the model

By Regina McEnery

The first humanized mice were created more than two decades ago. There are now four major types of humanized mouse models being used to study an array of infectious diseases, not least HIV. Though primates are still considered the best model for such research, humanized mice have the advantage of being far less costly. They have thus been used to test new HIV drugs and the systemic delivery of neutralizing antibodies against the virus (see *Mighty Mice*, *IAVI Report*, Sep.-Oct. 2008). Scientists have also designed humanized mice that appear to recapitulate the persistence of HIV in reservoirs of latently infected $CD4^+$ T cells. Such mice are likely to prove valuable to HIV cure research.

But they have so far proved to be less useful to HIV vaccine research, mainly due to limitations in their ability to generate functional T-cell responses against the virus that mimic those of humans. But four papers published recently suggest researchers have found a way around some of these barriers—most notably with the creation of the bone marrow-liver-thymus (BLT) humanized mouse. Those mice took a starring role at an all-day symposium at Harvard Medical School in Boston on Nov. 5 devoted to the application of humanized mouse models to AIDS vaccine development. "The immune responses in these models are very similar to what we see in human infection," said Todd Allen, co-chair of the symposium and principal investigator at The Ragon Institute of Massachusetts General Hospital (MGH), Massachusetts Institute of Technology (MIT), and Harvard. "But we don't know yet how well that will play out following vaccination of these mice. The biggest limitation is that this remains a model of a human immune system in a mouse environment."

A flurry of findings

Allen led a recent study that caused a small stir in AIDS vaccine research circles. He and his colleagues found that BLT mice infected with HIV mounted cellular immune responses that closely mirrored those observed in HIV-infected humans. HIV also escaped from those responses in a manner that generally mimicked natural infection (*Sci. Transl. Med.* **4**, 143ra98, 2012). Researchers detected HIV-specific cellular immune responses in the BLT mice, both through stimulation of epitope-specific T-cell lines and directly, *ex vivo*. Allen's team also found that BLT mice expressing the protective HLA-B*57 allele suppressed the virus in a way that was almost identical to how humans who express the same gene control the virus. They mounted responses against conserved regions of HIV Gag that are associated with greater control of viral replication in humans. Allen's lab is now looking at the potential to induce human HIV-specific immune responses in the humanized mice through vaccination.

Though mice are much smaller than people, they can shed light on how HIV makes its way around the body. This was vividly illustrated by Allen's Harvard colleague Thorsten Mempel at the mouse symposium. Mempel and his team recently tracked HIV-infected human T cells in the lymph node of a humanized mouse using a high-tech tool called intravital microscopy. This was the first time scientists have visualized the behavior of such cells in a live animal (*Nature* **490**, 283, 2012). The study found that HIV-infected T cells migrate robustly in lymph nodes, suggesting that their mobility facilitated the local dissemination of HIV infection in lymph nodes.

The study also found that in humanized mice

infected with an experimental strain of HIV that localizes to nuclei, the majority of elongated lymph node cells were multinucleated syncytia that likely developed through cell fusion. The uncoordinated motility of these syncytia and multiple adhesions to other CD4+ T cells in the lymph node resulted in the formation of continuous membrane surfaces that increased the effective length of infected cells some 10-fold. The researchers suggest that all this may facilitate cell-to-cell transmission of the virus and promote widespread HIV dissemination.

In yet another study, this one conducted by David Baltimore's lab at California Institute of Technology, scientists injected humanized mouse muscle cells with a modified viral vector optimized for the production of various broadly neutralizing antibodies. The researchers used vectored immunoprophylaxis (VIP) which utilizes a specialized adeno-associated virus (AAV) vector optimized for the production of full-length antibody from muscle tissue. The study found that antibodies delivered by VIP resulted in the long-lived production of antibodies in the mice. Further, the mice receiving VIP appear to be fully protected from HIV even when challenged intravenously with high doses of HIV. Alex Balazs, a researcher in David Baltimore's lab said at the mouse symposium that it remains to be seen whether the results seen in BLT mice can be replicated in humans. "History has shown us that humans don't behave like mice," said Balazs. "We have to be prepared for surprises."

A bone marrow-liver-thymus (BLT) mouse. *Jen Torrance/The Jackson Laboratory*

Humanized mice are contributing to research on novel therapies as well. Rockefeller University scientist Michel Nussenzweig has been testing cocktails of potent bNAbs as a therapy in humanized mice infected with HIV. He and his team have found that giving a single bNAb or even as many as three did not produce durable results; the virus rebounded weeks after the antibody treatment ceased. But when they increased the number of bNAbs to five, the virus had still not rebounded in seven of the eight mice after two months (*Nature* **492**, 118, 2012). Researchers suspect that the expanding arsenal of more potent antibodies might improve the chances of passive antibody transfer working and, if so, might provide an alternative to the daily grind of antiretroviral therapy. Instead of a BLT mouse, researchers used one that was a cross between a severe combined immune deficiency mouse and a non-obese diabetic mouse.

The evolving humanized mouse

The BLT mouse was initially developed by virologist J. Victor Garcia-Martinez, who is now at the University of North Carolina, in conjunction with a team at the University of Minnesota. Scientists make the mice by surgically implanting them with human organoids, which are fetal liver and thymic tissue that mimic organs—in this case organs that are essential to the development of immune cells. The mice are then irradiated and given transplants of stem cells taken from human fetal livers. These cells take up residence in the bone marrow, establishing a source for the human immune system borne by BLT mice. Mice altered this way were found to have a wide range of human immune cells in their peripheral blood; the cells also infiltrated tissues and organs in the lungs, GI tract, and liver, just as they would in the human body.

But the transplanted BLT immune system is not identical to a human's. For example, antibody-producing B lymphocytes don't mature properly in these mice. Dale Greiner, a University of Massachusetts scientist who has authored two reviews on the impact of humanized mouse models on the study of human disease, said this may be because the lymphoid organs in such mice are disorganized. It is in these organs that the immune responses are amplified and refined, especially those involving the production of neutralizing antibodies—which are today a major focus of HIV vaccine research.

In humans, he said, all of the components are "where they need to be." In humanized mice, "it is like walking into a warehouse, where everything is scattered." Greiner said that the genetic engineering required to remove the immune system in these mice, so that it can be replaced by a human one, might inadvertently disrupt the genes required to "organize" their lymphatic system in an immunologically functional manner.

Still, researchers are optimistic about the future of humanized mice in AIDS vaccine research. "What I think would really catalyze the field," said Andrew Tager, a Harvard Medical School scientist who collaborated with Allen on his recent study, "is if there could be funding for a consortium to focus on making this a better model with an eye toward answering more questions about HIV. How can we make the immune responses of the model even better? We have shown we are on track. The time is now." ■

Research BRIEFS

A surprising link between a staph toxin and CCR5

A research project that began by asking how the bacterium *Staphylococcus aureus* wreaks havoc in the human body found one of its answers in an unexpected place—a cell-surface receptor that a handful of pathogens, among them HIV, exploit to enter target cells. The study, led by New York University (NYU) researchers, showed that a soluble, bivalent pore-forming toxin called leukotoxin ED, or LukED, which *S. aureus* secretes to kill immune cells, appears to rely on the presence of the CCR5 protein to carry out the lethal act (*Nature* 2012, doi:10.1038/nature11724). HIV, *Toxoplasma gondii* and poxviruses are known to exploit CCR5 to target immune cells. But this appears to be the first time that *S. aureus* has been linked to the cell-surface protein.

This is of particular importance because antibiotic-resistant strains of *S. aureus* are a leading source of sometimes lethal infections contracted in hospitals. "In fact, in the US, *S. aureus* has been found to kill more people than HIV," says Victor Torres, assistant professor in the microbiology department at NYU School of Medicine and a co-author of the paper. "*S. aureus* is in every single hospital in the country. It's a major problem." The link between CCR5 and LukED could prove to be of some medical significance: an antiretroviral drug that disrupts HIV docking with this co-receptor is in widespread use today, raising the possibility that one means of dealing with drug resistant infections by *S. aureus* may already exist in the pharmacopeia.

The HIV co-receptor came into focus as a possible target of the bacterium when *in vitro* experiments conducted by Torres and his colleagues showed human T-cell lines expressing CCR5 died when exposed to a recombinant form of LukED, while Jurkat T-cell lines with undectable CCR5 were insensitive to the bacterial toxin. The cell lines came from the laboratory of Derya Unutmaz, associate professor of microbiology, pathology and medicine at NYU School of Medicine and a co-author of the paper. The picture became even clearer when a human osteosarcoma cell line engineered to express CCR5 was found to be sensitive to LukED, but not to other leukotoxins secreted by *S. aureus*.

Torres and his colleagues further found that the CCR5 antagonist maraviroc also blocked LukED's ability to kill CCR5$^+$ T cells *in vitro* at concentrations similar to those required to block HIV infection. This suggests that the antiviral drug might have some value in treating *S. aureus* strains that produce LukED toxin.

Mouse studies conducted by the team revealed that while mice lacking the CCR5 gene are largely resistant to infection with LukED$^+$ *S. aureus*, wild-type mice are highly susceptible to the infection. Additionally, primary murine macrophages treated with high concentrations of maraviroc were partly protected from toxin-mediated killing, further evidence that LukED directly targets mouse CCR5. Indeed, the researchers nailed down CCR5$^+$ effector memory T cells, macrophages, and dendritic cells as the preferred targets of the bivalent toxin, a clue as to why the *S. aureus* bug is so brutal. "CCR5$^+$ memory T cells secrete the cytokines (IL-17 or IFNg) that orchestrate the combat of the immune system against staph," says Unutmaz. "Targeting these cells is a quite ingenious way to knock down the command center of the adaptive immunity."

Torres says maraviroc could potentially be used as an adjunct treatment for staph. LukED-producing strains have been isolated from patients at a rate of 78%-95% depending on the study and site of infection. "Maraviroc has the potential of boosting immune cell survival, which will aid the host in controlling the bacterial infection." Mary Carrington, a senior investigator at the National Cancer Institute and SAIC in Frederick, who was not involved in the study, described the paper as "richly exciting" and agreed that it presents some "excellent possibilities for treating *S. aureus* infection."

The LukED findings also raise some intriguing questions relevant to HIV, notably whether *S. aureus* leukotoxins may have influenced the selection of the CCR5Δ32 allele associated with HIV resistance in rare individuals. NYU's Unutmaz thinks it might be worth studying whether individuals who carry the mutation are also relatively resistant to *S. aureus*. A proportion of individuals of Northern European heritage harbor the mutation, but when it surfaced, or why, is unclear. One theory was that it evolved in response to the Black Plague.

"We have no data to show that *S. aureus* [influenced selection]," says Torres. "What is intriguing is that *S. aureus* has been around humans for thousands of years, and at least in animal models, the pathogenesis of LukED$^+$ strains is primarily elicited in a CCR5-dependent manner."

But Carrington, who has studied cohorts of individuals who carry CCR5Δ32, says it isn't entirely clear what accounts for the persistence of the allele. "If the mutation was indeed selected by resistance to some deadly pathogen, then we would expect that pathogen to have been particularly devastating a few thousand years ago, when the mutation arose in Northern Europe, where the frequency of the delta32 allele is highest," she says. "That is why the plague was such a popular candidate for the driving force. But I don't know of any data that this was the case for *S. aureus*, but who knows. Even Icelandic health records were not so complete at that time." —*Regina McEnery*

Vaccine BRIEFS

Changes at The Global Fund and the NIH's Vaccine Research Center


Mark Dybul

The Global Fund to Fight AIDS, Tuberculosis and Malaria has picked Mark Dybul as its new executive director.

The appointment comes at a particularly rocky time for the Geneva-based organization, which has been grappling with both funding and management problems in recent years (see *The Global Fund's Uncertain Future*, *IAVI Report*, Jan.-Feb. 2012). A medical doctor and immunologist who helped create and then led the President's Emergency Program for AIDS Relief (PEPFAR) for three years, Dybul replaces Michel Kazatchkine, who left the organization in early 2012. Prior to Kazatchkine's departure, The Global Fund's board of directors appointed international banker Gabriel Jaramillo to the newly created position of general manager and put him in charge of day-to-day operations.

Dybul's appointment also comes at an important juncture in the global campaign against HIV. According to the latest Joint United Nations Programme on HIV/AIDS (UNAIDS) report, released Nov. 21, there are now 25 countries reporting at least a 50% drop in new infections over the past year (www.unaids.org). But progress remains a bit spotty. The number of people newly infected in the Middle East and North Africa has, for example, increased by more than 35% since 2001, and incidence has dramatically climbed in Eastern Europe and Central Asia.

Dybul was a staff clinician at the US National Institute of Allergy and Infectious Diseases (NIAID) when he joined a task force that led to the creation of PEPFAR in 2003. The following year, he joined PEPFAR as deputy chief medical officer, and in 2006 was named U.S. Global AIDS Coordinator, overseeing PEPFAR. Since 2009, he has co-directed the Global Health Law Program at the O'Neill Institute for National and Global Health Law at Georgetown University.

Mark Harrington, executive director of Treatment Action Group, an AIDS research and policy group in New York, said in an email that he was happy about Dybul's selection. "He is smart as a whip, his memory for data and science is outstanding, and he knows the players on the ground in many countries as a result of his years with PEPFAR."


Gary Nabel

News of Dybul's appointment was quickly followed by the announcement that the director of the Vaccine Research Center (VRC) at NIAID has joined Paris-based pharmaceutical giant Aventis as senior vice president and chief scientific officer. Gary Nabel, who joined the company on Dec. 3, will be based in Cambridge, Mass.

Nabel says the move was for personal reasons. His wife, Elizabeth, left her position as director of the US National Heart, Lung and Blood Institute in 2009, and is today president of Brigham and Women's Hospital in Boston. "We've been commuting for three years and, quite honestly, if it hadn't been for that, I wouldn't have looked for a new job in the first place," Nabel told *Science* writer Jon Cohen.

Nabel took the helm at the VRC when it was established 13 years ago, and NIAID's Executive Director Anthony Fauci said he will be difficult to replace. "Dr. Nabel's scientific contributions to ending some of the world's worst infectious diseases and causes of human suffering are extraordinary," said Fauci in a statement. "While I will continue to value his friendship, I will greatly miss his leadership and counsel here at NIAID."

Among the noteworthy achievements of the VRC during Nabel's tenure has been the identification in 2009 of several broadly neutralizing antibodies against HIV, including VRC01, which neutralizes 90% of a panel of Tier 2 viruses. The Center also conducted vaccine trials for Ebola, Marburg, West Nile, H5N1 avian influenza, and SARS viruses during his tenure. —*Regina McEnery*

Malaria vaccine candidate appears less effective in infants

New findings from an ongoing Phase III malaria vaccine trial in Africa suggest that the candidate, RTS,S, reduces the incidence of clinical malaria and severe malaria by a modest 31.3% and 36.6%, respectively, among children 6-12 weeks of age (*N. Engl. J. Med.* doi: 10/1056/NEJMoa1208394, 2012).

The efficacy of RTS,S in this group was less than that reported last year for older children enrolled in the same trial (*N. Engl. J. Med.* **365**, 1863, 2011). In the older children, ages 5-17 months, the RTS,S vaccine candidate was found to reduce the incidence of clinical malaria and severe malaria by 55.8% and 47.3% respectively. And results of a randomized, open label Phase II trial published last year found that vaccine efficacy against clinical malaria was as high as 61.6% among infants (*Lancet Infect. Dis.* **11**, 741, 2011).

Mary Hamel, a medical epidemiologist at the US Centers for Disease Control and Prevention and a principal investigator at one of the trial's clinical research centers in Kisumu, Kenya, said researchers should gain some clarity when data from all the sites where the study was conducted are released in the next year or two. "We may find that by pooling the data across the 11 trial sites, differences in vaccine efficacy by malaria transmission intensity were masked," Hamel says. "Most malaria cases in this analysis were from areas of very high transmission. Efficacy in areas of low or moderate malaria transmission may be higher, consistent with the Phase II trial."

Still, the results from the Phase III trial of the RTS,S vaccine candidate are considered a major milestone, given the unusual challenges of designing a vaccine against the malaria parasite, which is spread from human to human through the bite of infected *Anopheles* mosquitoes. The parasites travel through the bloodstream to the liver, where they mature and release another parasitic form, the merozoites, which then enter the bloodstream and infect red blood cells. The parasites largely live inside cells, where they avoid the body's immune responses, and humans do not develop sterilizing immunity against the pathogen. This means recurrent infections are common, at least in the developing world, where infected mosquitoes abound.

Developed and manufactured by GlaxoSmithKline (GSK) Biologicals, RTS,S contains a protein found on the surface of the *P. falciparum* sporozoite—the form of the parasite transmitted from mosquitoes to people—linked to hepatitis B vaccine antigen. It is formulated with AS01, an adjuvant manufactured by GSK.

The RTS,S candidate was co-administered with two licensed vaccines: a pentavalent vaccine against diphtheria, tetanus, pertussis, hepatitis B, and *Hemophilus influenzae* type B, and a polio vaccine. Scientists suggest that the co-administration of the licensed vaccines—including the Hep B antigen, which was effectively delivered twice—may have compromised the immune response to the RTS,S candidate. Hamel adds that infants have immature immune systems that respond less vigorously to vaccination, and that their responses might have been further compromised by antibodies against the sporozoites passed down by their mothers. Lower vaccine efficacy could also be associated with higher-transmission regions, but that will only be known when the site-specific analysis is completed.

The RTS,S candidate has been in development for nearly 30 years and would likely not have progressed this far were it not for the Malaria Vaccine Initiative (MVI), created by PATH, a Seattle-based non-profit established 30 years ago. PATH launched MVI in 1999, with an initial US$50 million grant from the Bill & Melinda Gates Foundation to accelerate the development of a malaria vaccine and ensure its accessibility in developing countries. MVI formed a product development partnership with GSK in 2001 to develop RTS,S.

The fate of RTS,S remains unclear. MVI, which financed most of the research with a $200 million grant from the Bill & Melinda Gates Foundation, hasn't yet announced any decision. "The efficacy came back lower than we had hoped, but developing a vaccine against a parasite is a very hard thing to do," said Bill Gates in a statement on PATH's website. "The trial is continuing, and we look forward to getting more data to help determine whether and how to deploy this vaccine." —*Regina McEnery*

Continued from page 7

charide vaccines such as PneumoVax, don't work in newborns because their immune systems are immature—featuring incompletely developed lymph nodes, for example, and lacking certain elements of the complement response.

To address this issue, Levy and colleagues are working on vaccines for newborns based on polymerosomes, 100-150 nm sized micelles formed by molecules that have both hydrophobic and hydrophilic segments. Because DCs are critical to a good vaccine response, Levy wants to test vaccines in an *in vitro* cell culture system that contains neonatal monocyte-derived DCs (MoDCs), which come from cord blood monocytes.

Another reason many vaccines don't work in newborns is that their immature APCs, such as DCs, are not easily activated. But Levy presented preliminary results suggesting that this barrier might be overcome with the use of his polymerosomes. He found that the nanoparticles are taken up by the cultured neonatal MoDCs, and even induce a cytokine response, which becomes stronger if the polymerosomes contain the TLR7/8 agonist resiquimod (R-848).

Next, Levy wants to develop a polymerosome-based HIV vaccine for newborns, in part because birth is the most reliable of relatively rare points of contact people have with health care providers in many developing countries. "In Africa, if you want to get immunizations into the population, it's going to be at the point of birth," he said.

He plans to put the HIV Gag protein and resiquimod inside the polymerosomes, and test whether that can induce antigen-specific immune responses in an *in vitro* culture system that simulates immunization of newborns. The culture will contain the neonatal MoDCs that take up the polymerosomes, and lymphocytes taken from cord blood of the same newborns that served as the source for the MoDCs. This way, Levy said, he can test if the polymerosome vaccine can induce lymphocyte proliferation and transition to a memory cell phenotype. If this works, Levy plans to test the system in monkeys.

When asked about possible safety concerns in newborns, Levy conceded that there is a higher safety bar when developing vaccines for newborns. Still, he said, the good safety and efficacy track record of BCG, a live-attenuated vaccine that activates multiple TLRs and is commonly given as a neonatal vaccine to prevent tuberculosis, provides some proof of concept and reassurance regarding safety issues. "When I started talking about this topic seven years ago," Levy said, "people used to tell me 'are you crazy? You are going to give a TLR agonist to a newborn?' Well, guess what, BCG activates TLR2, 4 [and] 8. So on a daily basis all over the world, newborns are being injected with Toll 2, 4 and 8 agonists." ■

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