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HAVI Report

Contents

Toll bridge to immunity

Immune molecules hold promise for vaccine adjuvant discovery

by Mary Lee MacKichan

Vaccine researchers have long depended on ill-defined additives called adjuvants to potentiate immune responses to immunogens. Injections of even large amounts of foreign protein from a pathogen are rarely enough to register on the radar of an animal's immune system. But add a pinch of an ingredient to the vaccine that bears no relation to the pathogen's protein—say mycobacterial cell wall extracts or alum—and the immune system springs to life. The late, great immunologist Charles Janeway famously termed the reliance on adjuvants to switch on the immune system the vaccinologist's "dirty little secret."

The pursuit of this mystery led to the discovery that some adjuvants work their magic on the immune system by activating Toll-like receptors (TLRs), now a red hot topic in immunology and vaccinology. They are the sentinels of the innate immune system, immediately sounding the alarm when a microbial invasion is detected. Crucially, TLRs, notably those on dendritic cells (DCs), also serve as a bridge to the adaptive immune system and the antigen-specific responses a vaccine elicits. The innate immune system and TLRs are now appreciated as principal actors in the drama between pathogen and host and their study will likely provide important lessons for vaccine design, including ones for the prevention of AIDS. "The beauty is that different TLRs seem to be specialized in the quality of the immune response they generate" says Bali Pulendran at Emory Vaccine Center. "This is great from a vaccinologist's point of view because it gives you new ways to manipulate the immune response."

TLRs are an evolutionarily ancient protein family. Toll, the founding member, was originally identified in the fruit fly *Drosophila* as an essential embryonic patterning molecule which is also required for innate defense against fungal infection. Human TLR genes were soon identified based on their sequence similarity to Toll, but a function in mammalian immunity was not demonstrated until Bruce Beutler from the Scripps Research Institute showed that in mice normal inflammatory responses to bacterial lipopolysaccharide (LPS) required TLR4. From there the linking of TLR family members with their microbial ligands proceeded quickly. To date 11 mouse and 10 human TLRs have been identified and shown to recognize a growing list of diverse molecules (Table 1; see *Nat. Rev. Immunol.* **4**, 499, 2004 for a more exhaustive list).

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Vaccine research lines converge

Vaccinologists working on different diseases met in Germany to exchange ideas—and found that their approaches already have a great deal in common

by Philip Cohen

Experts gathered at the Vaccine Congress 2005 in Berlin this September to compare notes on their progress battling many diseases around the globe. The meeting was titled *New Approaches to Vaccine Development: From the bench to the field* (www.vaccine-berlin2005.org). Over three days, speakers talked about challenges facing vaccine research, development, and delivery, from basic science to clinical trials as well as regulatory and safety issues. The infectious agents under the spotlight ranged from relatively new emergent threats such as the SARS-associated coronavirus, to the ongoing battle against major killers like HIV, tuberculosis, and malaria, to the endgame health officials are engaged in to eradicate poliovirus outbreaks from the planet.

Within those talks, the scientific tales told were impressive not only for the cutting edge biology on display but also for the extent to which different areas of research are converging, says Congress President Stefan Kaufmann of the Max Planck Institute for Infection Biology in Berlin. Vectors first developed for

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TLRs are expressed by nearly all mammalian cells and are now recognized as the first switch that needs to be thrown to initiate protective immune responses against pathogens. TLRs serve their role as immunological watchdogs mainly by recognizing highly conserved molecules comprising critical structures of bacteria, fungi, or viruses. Many of these structures, such as cell wall constituents, are essential to the microbe's survival or infectivity, making it difficult for the microbe to go undetected by a host immune system or for the microbe to evolve effective replacements. Other molecules recognized by TLRs, notably single-stranded RNAs, are not unique to pathogens and instead the distinction between self and non-self depends on their subcellular localization. TLRs recognizing such molecules are situated so that they come into contact with their ligands only when the source is a pathogen rather than the host.

Within minutes of activation, TLRs contribute to the innate immune response by triggering the release of inflammatory cytokines and inducing the antimicrobial activities of macrophages, including migration, phagocytosis, and direct killing. Through their effects on DCs in blood and tissues, TLRs also lead to the induction of adaptive immunity, acting on both the myeloid DC (mDC) and plasmacytoid DC (pDC) subsets. When DCs encounter TLR ligands in tissues, they are stimulated to increase antigen uptake, migrate to lymph nodes and mature, up-regulating expression of MHC and co-activator molecules. Such mature DCs are required as antigen-presenting cells (APCs) that activate naive $CD4^+$ T lymphocytes to become helper cells and differentiate into memory cells. Another APC type, B lymphocytes, is affected both indirectly and directly by TLR signaling: cytokines from $CD4^+$ T cells influence antibody affinity and class switching, and B cells undergo polyclonal activation in response to some TLR ligands, including LPS (see *Research Briefs*, this issue).

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Anatomy of TLR

TLRs are membrane spanning proteins, many of which function on the surfaces of cells, while others work in internal cell compartments. The ligand recognition domain of TLRs is comprised of leucine-rich repeats (LRRs) and serves as the antenna which detects microbial molecules. Though not yet conclusively demonstrated, data suggest these repeats bind ligand directly. Additional co-receptors can be involved in ligand recognition, as is the case for TLR2 (CD36) and TLR4 (CD14 and MD2), while as yet unidentified co-receptors may interact with other TLRs.

The signaling domain of a TLR transmits news of microbial invasion to cells through a classical signal transduction pathway, comprised of a complex set of molecular relays. This domain consists of a Toll–IL1 receptor (TIR) element, so called because it is also present in the receptor for the pro-inflammatory cytokine interleukin-1. Revealing the mechanism of how TLR activation is translated into immune function is a rapidly moving area of research. What's clear is that the signaling is mediated in large part by the cytosolic adaptor protein MyD88, a protein that has its own TIR domain that directly engages its twin on TLRs. Through a separate domain, MyD88 then recruits signaling molecules, including IRAK family kinases and TRAF6, to turn on downstream

TLR	Typical Ligands
TLR1	Bacterial lipoproteins
TLR2	lipoteichoic acid (LTA), peptidoglycan (PGN), bacterial lipoproteins, zymosan
TLR3	Double-stranded RNA
TLR4	Lipopolysaccharide (LPS), viral envelope protein MMTV, RSV F protein
TLR5	Flagellin
TLR6	LTA, diacyl lipoproteins, zymosan
TLR7	ssRNA, imidazoquinolines
TLR8	ssRNA, imidazoquinolines
TLR9	Unmethylated CpG DNA
TLR10	Undetermined
TLR11*	Uropathogenic bacteria, profilin-like protein

** a functional gene for TLR11 has only been found in mice.*

Table 1. Toll-like receptors (TLRs) and some of their important ligands. *(Adapted from Akira and Takeda,* Nat. Rev. Immunol. ***4****, 499, 2004.)*

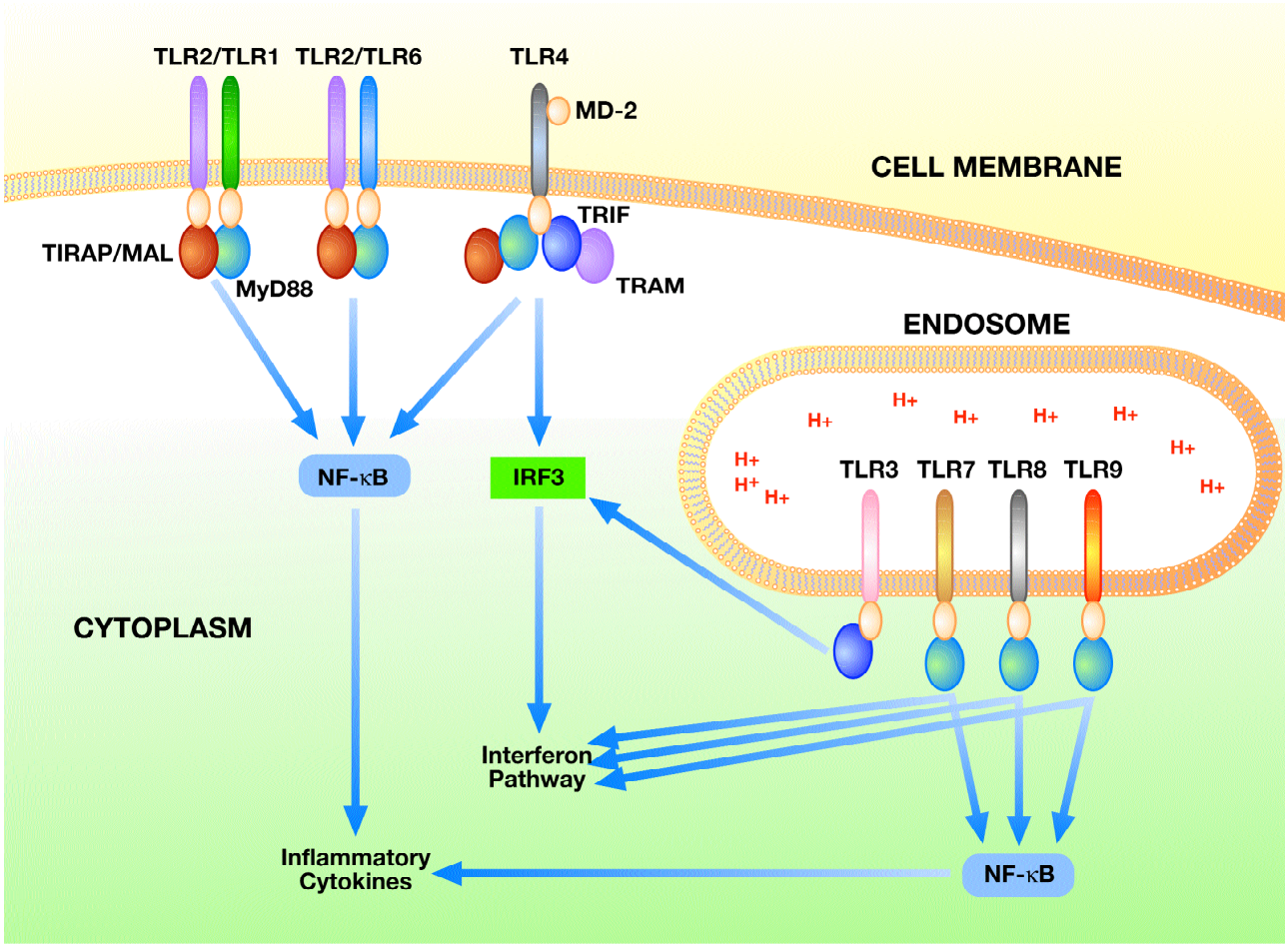

Figure 1. TLR signaling pathways. *TLRs are transmembrane proteins that act alone or in heterologous pairs to detect invading microbes, triggering signaling pathways that ultimately lead to the release of inflammatory cytokines through the NF-κB pathway or the release of interferons through the IRF3 pathway. TLRs can localize to the cell membrane or endosomes and can differ in terms of the adaptor molecules (circles) that relay their intracellular signals. Some of the adaptor molecules have been characterized (MyD88, TRIF, TRAM, TIRAP/MAL), but others remain undefined (adapted from Boehme and Compton,* J. Virol. ***78****, 7867, 2004).*

pathways leading to activation of MAP kinases and the transcription factors NF-κB and interferon response factors (IRFs). TLR3 signaling is uniquely independent of MyD88 and instead relies on another adaptor, TRIF, to link it to downstream pathways. Activation of these pathways ultimately leads, depending on the TLR and cell type, to expression of cytokine and co-stimulatory molecules, cellular proliferation or increased survival, and changes in actin and cell motility (Figure 1).

Microarray studies of TLR-induced gene expression confirm that different TLRs modulate expression of common target genes while other genes are specifically responsive to a given TLR. Differences in adaptor protein recruitment can account for some of the specificity of responses to different TLRs. But other subtle differences in the signaling cascades downstream of TLRs can also translate into large differences in gene transcription and ensuing adaptive immunity. For example, initial signaling responses following activation of TLR2 and TLR4 overlap significantly. But Pulendran's laboratory has demonstrated that DC activation following engagement of TLR2 results in Th2 $CD4^+$ helper T cell responses, while TLR4 results in Th1 responses. Further investigation revealed that the basis of this difference lies in the quantitative, rather than qualitative, aspects of the initial response: the TLR2 ligand induced more prolonged and intense activation of the signaling molecule ERK MAP kinase than did the molecule binding TLR4. The enhanced ERK signaling downstream of TLR2 stabilized the transcription factor c-Fos which, in turn, repressed IL-12 expression by DCs, resulting in a Th2 response (*J. Immunol.* **171**, 4983, 2003).

Antiviral responses

While the detailed roadmap of the links between TLRs, downstream pathway activation, and immune responses is still being drawn, the importance of TLR 3, 7, 8, and 9 for antiviral immunity is emerging rapidly (reviewed in *J. Virol.* **78**, 7869, 2004). All four receptors recognize nucleic acids and endosomal acidification is required for their activation. It is believed that viral ligands come into contact with TLRs in APCs through receptor-mediated uptake of virus or viral fusion with endosomal

membranes (see *Proc. Natl Acad. Sci. USA* **101**, 6835, 2005 for discussion). For viruses that either do not infect or cannot replicate in APCs, $CD8^+$ T cells can be activated by cross-priming from DCs that engulf and then present antigen from apoptotic cells infected with virus. TLR3 in APCs appears to be involved in mediating such cross-priming by recognizing double-stranded RNAs found in infected apoptotic cells. A recent report in *Nature* (**433**, 887, 2005) showed that the robust cytotoxic T lymphocyte (CTL) response produced by immunization with virally-infected cells required this receptor and phagocytosis.

Interferon-α (IFN-α) is a hallmark of the response to many viral infections and pDCs—which express TLRs 7 and 9—are the major source of this cytokine (see *J. Virol.* **79**, 17, 2005 for review of pDCs). IFN-α stimulates multiple innate protective pathways, including intracellular RNAse activity, that inhibit viral replication and can lead to viral clearance. Human pDCs have been shown to be activated via TLRs to make IFN-α in response to multiple enveloped viruses, including influenza virus and HIV.

TLRs and HIV

The role of TLRs in the HIV-specific immune response is slowly being unraveled. Multiple studies support the idea that HIV nucleic acids, and possibly envelope protein, are involved in activating TLRs during infection. A ssRNA oligonucleotide derived from HIV-1 U5 was shown to act through TLR8, and possibly TLR7, to induce production of IFN-α and other cytokines (*Science* **303**, 1526, 2004). In addition to evidence that synthetic HIV sequences can be detected by TLRs, human pDCs are activated and mature following exposure to intact HIV *in vitro* (*J. Virol.* **78**, 5223, 2004). This pDC response to HIV is unaffected by chemical inactivation of the virus, suggesting that membrane fusion or replication is not required for detection by TLRs. Nina Bhardwaj's laboratory at New York University recently demonstrated that viral nucleic acid is required for HIV stimulation of pDC through TLRs and identified TLR7 as the likely target for recognition of HIV RNA. However, HIV uptake, mediated by envelope protein and cellular CD4 interactions, as well as subsequent endosomal acidification, are also required for viral RNA to reach TLR7 (*J. Clin. Invest.* **115**, 3265, 2005).

While HIV clearly can activate pDCs via TLRs *in vitro*, during natural infection this interaction may fail to occur or the virus may interfere with downstream responses allowing establishment of a chronic infection. Precedent for such interference has been uncovered in other viral infections, including vaccinia and hepatitis C virus (HCV). Vaccinia encodes a TIR-containing decoy protein that blocks TLR signaling (*J. Exp. Med.* **201**, 1007, 2005), while *in vitro* evidence suggests HCV NS3/4 protease can cleave TRIF to suppress TLR3-mediated production of IFNs and other cytokines (*Proc. Natl Acad. Sci. USA* **102**, 2992, 2005). If it turns out that HIV latency or chronicity involves interference with TLR pathways, any such mechanism could offer a novel target for intervention.

TLRs and vaccines

The goal for vaccinologists in tapping TLR responses is to strike a balance between immune activation and the potential damage that can result from inflammation. The importance of TLR-mediated responses in achieving protective immunity is being reinforced by research from the Pulendran laboratory where he and his colleagues have begun analyzing the workings of the highly efficacious yellow fever vaccine, YF-17D. Their results suggest YF-17D activates multiple TLRs, including TLR 2, 7, 8, and 9 on multiple subsets of DCs. "It's almost too good to be true. The vaccine induces a remarkably broad spectrum of responses including CTL, broad neutralizing antibodies, Th1, and Th2 responses. It is clear that this diversity is achieved by activating multiple TLRs," says Pulendran. "I think YF-17D might be teaching us vaccinologists a lesson, providing a clear scientific rationale for incorporating more than one (TLR) ligand into a vaccine formulation." However, he notes that the need to license multiple TLR ligands could be a hurdle to commercialization of this approach.

As with any attempt to tinker with natural responses, manipulating the immune system via TLRs may have a dark side. Because TLRs recognize nucleic acids some experts have proposed a role for TLRs in the generation of lupus-like autoimmune syndromes characterized by the production of anti-DNA and -RNA antibodies. TLR activation has also been suggested to be involved in the genesis of asthma and allergy. In addition, a polymorphism identified in human TLR4 not only correlates with increased susceptibility to certain bacterial infections but also to a lower risk of atherosclerosis and acute cardiac events, suggesting that activation of other TLR4 alleles could promote atherosclerosis (see *Nat. Immunol.* **5**, 975, 2005 for a review of TLRs in disease.) "The key for vaccines will be to harness immunostimulatory parts of the pathways and limit the toxic parts of the pathways," says Richard Ulevitch of

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Scripps Research Institute. Robert Seder at the US National Institutes of Health believes some "side-effects" will be unavoidably associated with any TLR agonist. "These are inflammatory responses," he says. "People are going to get feverish." Pulendran suggests correlates of immunity versus toxicity may be defined by thresholds rather than distinct pathways. "It may be that just more of the same thing is bad," he says. He emphasizes the need for quantitative experiments with titrated doses of adjuvants to separate out beneficial effects from toxicity.

One complication to development of novel TLR ligands for use in human vaccines is the species-specificity of TLR expression, ligand recognition, and DC subsets. Mice can make a robust immune response that includes CTL in response to vaccination with TLR ligands such as CpG oligonucleotides and protein antigen, but the $CD8^+$ DC subset responsible may not be present in primates. Because of the limitations of mouse models to answer all questions surrounding TLRs, Susan Barnett of Chiron says her group uses multiple animal models as well as *in vitro* screens to test TLR-related vaccine adjuvants. But Barnett says that TLR ligands, such as CpG oligonucleotides, might be tested clinically as part of an AIDS vaccine in the next few years, allowing direct assessment of their efficacy in humans.

Using what is already known about TLRs, vaccinologists have begun shifting from a largely empirical approach to the more rational design of adjuvants to take advantage of these mechanistic insights. The investigation of microbial and synthetic TLR ligands already underway has intensified, with the majority of attention focusing on CpG oligonucleotides (for TLR9) and small molecules in the imidazoquinoline family. CpG oligonucleotides have received much attention for their potential to activate multiple cell types and promote Th1 responses and are currently undergoing testing in the clinic for treatment of cancer and viral infection. Imiquimod, a small molecule imidazoquinoline ligand for TLR7 and 8, is already marketed as a topical antiviral to treat human papillomavirus and basal cell carcinoma. The drug's activity was discovered empirically and later the mode of action was shown to involve TLR-dependent activation of DCs to secrete IFNs and other cytokines. With the TLRs in hand the search for new synthetic TLR ligands is now booming (see *Nat. Rev. Drug Discov.* **4**, 879, 2005 for a detailed summary of TLR-targeted therapeutics). While most of these efforts are focused on therapeutic applications, "in principle it should be possible to set up screens for TLRs that would identify novel ligands that could act as adjuvants" says Ulevitch.

Using drug-like chemicals as adjuvants may also have the advantage of allowing the TLR stimulus and antigen to be delivered together, possibly as conjugated molecules. "Synchronous delivery is going to be critical. If protein arrives after the TLR stimulus is gone it's a bad thing because DCs won't take it up," says Seder. "These (conjugated TLR ligand and protein antigen) are terrific for antibody and Th1 responses," he says, "and I think it is possible to generate $CD8^+$ T cell responses with proteins under the right conditions. I don't think they will be as good as replication-defective viral vaccines, but that doesn't mean they can't be used in combination with virus." While citing evidence from his own work (see *Research Briefs*, this issue) and others suggesting conjugation dramatically improves T cell responses, he cautions that conjugation could sometimes produce suboptimal antibody responses.

The full potential of harnessing TLR signaling pathways will only be clear once they are fully elucidated. And that work is, at best, half done. Based on saturation mutagenesis studies, Beutler calculates that approximately fifty proteins may have non-redundant function in TLR signaling to induce TNF, and quite a few more may be involved in IFN production. More than half of these proteins remain to be identified. Even so, he thinks that TLRs may not offer the best way to stimulate every aspect of immunity. "While TLRs give mainly a $CD4^+$ T cell response, other TIR-independent pathways induced by apoptotic cells are better inducers of $CD8^+$ responses." Pulendran cites other open questions in the field. "Understanding how TLRs interact with other receptors in the context of a real infection will be important. We don't yet know how to get a good B cell response, whether we need to engage TLR on the B cell itself in addition to APC activation. There is scope for a lot of research."

Looking ahead, Pulendran envisions a day when designer adjuvants will combine TLR ligands and small molecules to other targets to push the immune system in precisely the right direction for a given vaccine. "Although it sounds like science fiction, in 15 years or sooner adjuvants may be nanoparticles that contain not only specific TLR ligands but also specific inhibitors of intracellular signalers like ERK or c-Fos," he says.

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>
> **Bali Pulendran**

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HIV and TB are a dangerous liaison

Stefan Kaufman

one disease are now being eyed for multi-disease inoculations, for example, and interest in new adjuvants is soaring across the board. While such cross fertilization is not unusual in scientific research, Kaufmann thinks a sense of urgency has also driven researchers to look for new approaches in related and unrelated diseases. "The clock of infections is ticking, and we don't want to lose any more time," he says.

TB or not TB

About three billion doses of the live-attenuated vaccine for tuberculosis (TB), *Mycobacterium bovis* bacilli Calmette-Guerin (BCG), have been dispensed and have played a major role in protecting children from severe infections with *M. tuberculosis*. Even so this vaccine, first developed more than 80 years ago, needs improvement. BCG contains rather than eradicates *M. tuberculosis* infection, so doesn't prevent the most prevalent form of TB infection in adults, pulmonary tuberculosis; in some populations it appears to have little or no effect, which appears especially true in developing country settings.

The urgency for improved TB vaccines is increasing with the emergence of *M. tuberculosis* strains resistant to multiple drugs. And more and more people are becoming co-infected with *M. tuberculosis* and HIV making treatment further complicated since antiretrovirals can trigger TB outbreaks and BCG can cause disease in immunocompromised people. "HIV and TB are a dangerous liaison," says Kaufmann. But the reasons for poor protection afforded by BCG in some settings aren't yet clear. Failure may be related to the local mycobacterial strains and their effect on immunity, or interaction with BCG. It's also possible that genetic differences between BCG and *M. tuberculosis* or differences in their immunogenicity limit how effective vaccination can be.

Kaufmann began his efforts to improve BCG with an analysis of what limits its immunogenicity. When this mycobacterium invades antigen-presenting cells, like macrophages or dendritic cells (DCs), it takes up residence in vacuoles within cells called phagosomes. Bacterial protein antigens from this compartment are presented in the context of MHC II, which primarily stimulates $CD4^+$ T cells. As a result BCG antigens rarely enter the MHC I pathway, eliciting few $CD8^+$ T cells, which are known to be major contributors to TB immunity.

It was clear that the road to greater immunity lay outside of the phagosome. So Kaufmann's team set to build BCG antigens an escape vehicle. They genetically engineered recombinant BCG (rBCG) to carry a protein from *Listeria monocytogenes* called listeriolysin, which forms protein pores in phagosome membranes. Since this protein operates optimally at acidic pH, the investigators also knocked out the gene for BCG urease C, an enzyme that normally buffers the phagosomal environment to near neutrality, to create their ΔureC hly^+ rBCG strain.

Mice vaccinated with this new rBCG strain showed superior protection when challenged by *M. tuberculosis*. Most strikingly, animals vaccinated with rBCG and then challenged with the multi-drug resistant clinical *M. tuberculosis* isolate Beijing/W had a 100-fold reduction of bacterial load in their lungs compared to mice vaccinated with the parental BCG, which showed no significant protection above control vaccinations against this pathogen. The improved strain also had an increased safety profile; immunodeficient SCID mice survived 80 days after inoculation with 10^8 colony forming units of ΔureC hly^+ rBCG while the immunocompromised rodents receiving similar doses of BCG died in less than 25 days.

The rBCG vaccine does elicit $CD8^+$ T cells, including some with antigen-specific cytotoxicity. It turns out, however, that this improved immunogenicity is only partly due to the intended listeriolysin-aided leakage of rBCG antigens to the cytoplasm. Kaufmann's team found that the microbe also triggers profound levels of apoptosis when it infects mouse or human macrophages. *In vivo*, apoptotic cells would release vesicles with bacterial antigens which could be presented in the context of MHC I and MHC II after they are engulfed by neighboring DCs. How Δure C hly^+ rBCG triggers apoptosis is unclear, although Kaufmann suspects it may be initiated by the listeriolysin-aided release of cellular proteases from the phagosome. Such proteases are known to activate apoptotic signal cascades. The researchers have patented their rBCG strain and licensed it to Vakzine Projekt Management GmbH, which is preparing to start clinical trials at the end of this year.

Boosting BCG immunity by creating a leaky phagosome appears to be in vogue. In a later talk Jerry Sadoff of Aeras Global TB Vaccine Foundation reported that his team has developed a similar strategy using the pore-forming protein perfringolysin O from *Clostridium perfringens*. This protein has the advantage of operating at near neutral pH and thus not requiring the genetic background of a urease C mutation for optimal function. Sadoff's team is now in the process of generating immunogenicity data.

Viruses…good, bad, and bacterial

There aren't many examples of beneficial viral infections. Which is why one of the more intriguing mysteries about AIDS surrounds people co-infected with HIV and GB virus C (GBV-C)—a number of studies have suggested people with the dual infection have slower disease progression, higher $CD4^+$ T cells counts and survive longer than those infected with HIV alone, suggesting GBV-C affords some protection. Bemhard Fleckenstein and Heide Reil at Friedrich-Alexander-University Erlangen-Nümberg and their colleagues are investigating the mechanism of this protection by studying how the two viruses interact in peripheral blood mononucleocytes (PBMCs) in cell culture.

His team found that coinfection of human PBMCs with the viruses resulted in an average of 90% inhibition of HIV replication compared to single HIV infection. GBV-C inhibited all HIV subtypes and strains, including R5 and X4 isolates. Fleckenstein showed preliminary work suggesting that some of that inhibition is due to the GBV-C protein E2. When a recombinant version of this protein is simply added to the media of HIV-infected cells it inhibits HIV replication. Adding monoclonal antibodies against E2 protein abolishes this inhibition. It isn't yet clear what the precise mechanism of E2 inhibition is, how strain specific these effects are, or if other GBV-C proteins have similar anti-HIV activity. If E2 and other GBV-C proteins prove to be a powerful inhibitors of HIV activity, Fleckenstein speculates it may be possible to use them therapeutically.

For flu researchers, fears of a new pandemic are being fed by the recent discovery that the devastating 1918 pandemic strain was most likely an avian influenza that jumped to humans—a concern given the increasing reports of outbreaks of avian flu in humans. However little is actually known about how such pandemic strains form and cross the species barrier. To begin addressing this question, Hans-Dieter Klenk of Philipps University in Germany characterized the ability of avian and human flu viruses to infect human lung tissue. The flu virus protein hemagglutinin (HA) binds cells through sugar residues on surface proteins. The sugars recognized by human virus HA are 2-6 linked sialic acids while bird virus prefers 2-3 linked sialic acids.

The researchers infected cultures of human tracheo-bronchial eptihelium with the viruses using a system that maintains the morphology and function of the lung cells, including cilia beating. They found that 2-6 linked sugars are abundant on non-ciliated tracheo-bronchial cells and during initial infection human virus predominantly infects these cells. In contrast, 2-3 linkages were seen on ciliated cells in sufficient numbers to allow infection of these cells by avian flu. "Ciliated cells are the entry site of avian flu viruses into the human respiratory tract," says Klenk. However, the avian virus did not spread through the culture while the human virus reached high enough concentration to infect even ciliated cells containing the non-preferred receptor. This suggests that human lung ciliated cells may serve as a milieu where avian and human flus can recombine.

Mammalian viruses form the basis of many vaccines now in development. But in the search for new tools, David Hone and his colleagues at Aeras have reached further down the viral evolutionary ladder. In their system, the ϕ8 bacteriophage forms the basis of their delivery system for genetic sequences for the TB antigens 85A and 85B. The ϕ8 phage contains a double-stranded RNA genome in three segments: small (S), medium (M), and large (L). The researchers removed phage genes from the two smaller segments and substituted those for TB antigens. The L segment, which contains all sequences necessary for the construction of the viral nucleocapsid, was left intact. These recombinant nucleocapsids replicate to copy numbers of 150 to 200 inside *Shigella* bacteria.

The plan is to deliver the nucleocapid-carrying *Shigella* orally and have the bacteria invade macrophage and DCs. The bacteria naturally escape endosomes and are engineered with a cell wall defect so that they lyse as they try to divide, spilling the ϕ8 particles into the cytoplasm. There, finding themselves in a pool of ribonucleotides, the nucleocapsids spontaneously start spooling out single-stranded RNA that contains the antigen gene sequence from their interior, which is translated into protein by the host cell. Hone says they have already used the system in a mouse model to deliver the TB antigens. While preliminary, the results look promising. "We appear to be inducing IFN-γ at the same level as an adenovirus vector with the same antigens," he says. This hybrid bacterial/phage vector could potentially be produced at low cost and, since the antigen genes are never present as DNA, carries virtually no risk for chromosome integration, one concern for DNA-based vectors. His team is now planning to determine the strength and protectiveness of the response elicited by vector and characterize the T cell subsets that respond.

William Jacobs of the Albert Einstein College of Medicine in New York has attacked the problem of improving TB vaccination from the opposite direction. Rather than building up BCG, his team is stripping down wild-type pathogenic *M. tuberculosis*. In the early 20th century, BCG was attenuated by more than 200 serial passages in bile salts of a virulent bacterial strain resulting in the loss or rearrangement of more than 100 genes. Today, with the entire genome of *M. tuberculosis* sequenced and the ability to knock out genes at will, Jacobs is making progress toward reducing this microbe's virulence while preserving its immunogenicity by creating strains with designer combinations of mutations.

One approach Jacobs described is combining mutations in different biosynthetic pathways, resulting in slow growth *in vivo*. A promising mutant dubbed $mc^2$6020 contains deletions in genes for lysine and pantothenate synthesis and shows dramatic attenuation. When interferon-γ (IFN-γ) knockout (GKO) mice, which are very susceptible to TB infection, were inoculated with 10^5 colony forming units of this mutant all the animals survived after 400 days of observation. In contrast, mean survival times for BCG-infected GKO mice were less than 100 days.

When used as a vaccine, immunological protection provided by $mc^2$6020 was similar to BCG in a mouse model of TB challenge. But Jacobs is also working to improve immunogenicity of his strain. His team identified a gene called *NlaA* (Nurim-like anti-apoptotic factor), which when knocked out in *M. tuberculosis* strains causes more apoptosis in infected cells and therefore presumably better antigen presentation.

Along with their use as TB vaccines, the rBCG and mc^{2}6020 strains may be valuable as platforms for the delivery of other antigens. Jacobs mentioned that he is collaborating with Barton Haynes (see *An Interview with Barton Haynes*, page 14), team leader for the recently awarded CHAVI grant from the US National Institutes of Health (NIH), to explore using mc^{2}6020 as an AIDS vaccine. The collaborators are currently planning to test immunogenicity in mice, guinea pigs, cattle, and non-human primates.

Jaap Goudsmit of the Netherlands-based company Crucell also argued that rBCG strains would be valuable vaccine vectors for other diseases. His company collaborates with Aeras (which provides rBCG strains), IAVI, and the NIH to develop vaccines against TB, HIV, and malaria using its proprietary adenovirus vaccine vector serotype 35 (Ad35). "An rBCG vaccine followed by an Ad35 boost is the strategy of choice for combined pediatric vaccine against HIV, malaria, and TB," he says. Goudsmit thinks there are a number of arguments in favor of childhood vaccination against HIV: it could lower mother to child transmission of HIV, guarantee that individuals would be vaccinated before they became sexually active, and deal with the issue of pre-existing immunity against vectors. "We eventually will need to combine vaccinations or we'll run into problems since researchers for these different diseases are all using the same vectors," he says.

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Jaap Goudsmit

Bacterial balancing acts

The challenge in these attempts to supercharge bacteria as vaccine vectors is to strike the right balance between a hardy vector that stimulates immunity and one that poses no danger for its human host. "Ideally you want wild-type ability to withstand stress, wild-type ability to invade cells before the vector displays attenuation," says Roy Curtiss III of the Biodesign Institute at Arizona State University in Tempe. Curtiss reported on a clever genetic strategy to maximize the efficiency of *Salmonella* spp. vectors by placing virulence factors or other essential genes under control of an arabinose-inducible promoter. As a result, the bacteria can be grown in arabinose media with wild-type stress resistance and invasive powers, which wane after a few generations of growth *in vivo*. He showed one example of this regulated attenutation strategy in which the phosphomannose isomerase gene, which adds sugars to the protective surface LPS-O-antigen, was placed in this cassette. Growth *in vivo* causes the shut down of the isomerase gene in the arabinose cassette. As a result, new sugars are not added to the bacterial LPS-O-antigen and the *Salmonella* strain becomes highly attenuated after seven generations of growth. "We got high immunogenicity but some mice die," says Curtiss. To quicken the attenuation, Curtiss is looking to add more genes to the regulated cassette. One attractive set of candidates are genes that drive synthesis of the bacterial wall component diaminopimelic acid (DAP). Loss of DAP results in bacterial lysis, attenuating the strain and also increasing antigen delivery to the cell. Curtiss recently received a $14.8 million grant from the Bill & Melinda Gates Foundation's Grand Challenges in Global Health Initiative to develop a new pneumonia vaccine for newborns based on this technology.

Alexander Schmidt at the University of Muenster, Germany and his colleagues have avoided the attenuation question altogether by using a microbial vector that is not only safe but beneficial in some people. The probiotic *Eschericia coli* Nissle 1917 strain has long been used to treat inflammatory bowel disease and recently has received attention as a vector to deliver therapeutic molecules (*FEMS Immunol. Med. Microbiol.* **43**, 373, 2005). Oddly, the Nissle 1917 field isolate carries a number of genetic markers associated with pathogenic strains and is known to have adjuvant properties. "To a microbiologist and the immune system at first glance it may look like a bad guy, but behaves very well indeed," says Schmidt. "I've taken it myself. Besides tasting like *E. coli* it has no ill effects whatsoever."

To this strain Schmidt has added a recombinant version of the *E. coli* gene for AIDA-1 (Adhesin Involved in Diffuse Adherence), a protein transporter which delivers an adhesin to the surface of some strains. His team substituted the adhesin N-terminus of the protein for antigens from Shiga toxin of enterohemorrhagic *E. coli* (EHEC) and the OspA protein from the lyme disease bacteria *Borrelia burgdorferi*. After 8 to 9 days of oral immunization, the researchers were able to measure specific mucosal and systemic antibody responses against these antigens despite poor bacterial colonization of the murine intestine. His team is now working to develop disease models in the mouse to test for protection.

Adjuvant extravaganza

Another hot topic at the meeting was designing adjuvants and other strategies to give more kick to vaccines. Hermann Wagner at the Technical University of Munich described the use of microspheres to co-deliver adjuvant and

antigen. Wagner's team is hoping to extend the success his and other labs have had using ligands for Toll-like receptors (TLRs) chemically coupled to antigens to boost immune responses (see *Toll bridge to immunity*, page 1, and *Research Briefs*, this issue). "The problem is that the chemistry for these complexes must be optimized for each antigen, which isn't ideal," says Wagner.

So instead, Wagner's team took a solution containing the TLR9 ligand CpG and a model protein, ovalbumin (OVA), and trapped it inside polylactide microspheres. They found these microspheres are readily ingested by DCs and make their way to endosomes where TLR9 resides. In a proof-of-concept study, the spheres were injected subcutaneously into mice and the animals developed OVA-specific $CD8^+$ T cell responses, good antibody responses, and were protected against infection by a *L. monocytogenes* strain expressing OVA.

Annie Mo of Antigenics presented her company's strategy of inducing cellular immunity by complexing antigenic peptides with the heat shock protein HSP70. HSP70 contains a peptide-binding pocket and can act as a chaperone for antigenic peptides. It can also bind the CD91 cell surface receptor, facilitating peptide uptake by APCs through endocytosis. The peptides carried by HSP70 are then processed and presented in the context of MHC molecules. Antigenics has already developed a number of cancer immunotherapies now in clinical trials which involve purifying HSP70-peptide complexes from a patient's own tumors. But the ability of *in vitro* reconstituted HSP70-peptide complexes to induce strong immune responses to viral antigens has been demonstrated in animal models for antigens from influenza virus and HIV. Mo described her company's first HSP-based polyvalent vaccine program on an infectious disease, a therapeutic vaccine for herpes simplex virus type 2 (HSV-2).

The HSV-2 vaccine is composed of a species-matched recombinant HSP70 and synthetic peptides derived from HSV-2 proteins. To optimize the system, they used a 35-mer peptide from HSV-2 glycoprotein B that contains a known $CD8^+$ T cell epitope. Preliminary experiments showed that peptide concentrations as low as 0.02 nanomolar complexed with HSP70 elicited a measurable antigen-specific T cell response. And the specific response to the peptide improved more than 3-fold when it was just one of a peptide pool. "That was good news," says Mo. "It suggested a polyvalent peptide pool would work." Indeed, in a mouse model a polyvalent HSV vaccine delivered by HSP70 increases the frequency of IFN-γ secreting T cells 4-fold over peptide alone. After the meeting, Antigenics announced they have started a Phase I trial of a recombinant human HSP complexed with 32 synthetic peptides representing HSV-2 proteins.

A specific class of adjuvants gaining in interest are those that elicit mucosal immunity. Mucosal sites are a major point of entry for pathogens and recently have become a focus of AIDS research with the discovery that much of the crucial immunological damage wrought by HIV occurs in gut mucosa within the first two weeks of infection. One of the best studied and most potent mucosal adjuvants is cholera toxin (CT), but this molecule is also highly toxic making it impractical as a component of human vaccines.

Jan Holmgren at Goteberg University in Sweden is interested in developing non-toxic alternatives to CT. One approach he discussed was to isolate the B subunit of CT (CTB), a non-toxic molecule which, while less powerful than CT, can be used to promote mucosal immunity. Holmgren tried to further boost its effectiveness by linking recombinant CTB to CpG oligonucleotide that mimics bacterial DNA and engages TLR9. They found that in mice treated intravaginally the CTB-CpG conjugate elicited more than 10-fold stronger production of MIP-1α, MIP1-β and RANTES in the vaginal mucosa over each component separately or the two mixed together. There was also very strong expression of these chemokines by human lymphocytes exposed to the CTB-CpG conjugate. Their analysis shows the effect is dependent on TLR9, suggesting CTB helps to deliver CpG to this receptor in endosomes by binding its ligand, the GM1 receptor.

Holmgren, Cecil Czerkinsky's team of INSERM in France, and their colleagues also presented a poster on linking CTB to a model antigen, OVA, for use as a vaginal immunogen. In a mouse model, CTB-OVA applied vaginally activated antigen-specific IFN-γ secreting $CD4^+$ and $CD8^+$ T cells and elicited antigen-specific cytotoxic T lymphocytes. OVA or CTB delivered alone or mixed together failed to trigger a comparable immune response.

The site of delivery of mucosal vaccines is known to have a powerful effect in many mammals including mice, rats, macaques and humans. In general, the strongest IgA production is at the site of administration and then weakens in proportion to distance. Nasal application is the exception to this rule since it generates a strong immune response at distal sites including the vaginal tract. Holmgren and Czerkinsky reported on another promising delivery route: under the tongue. Using CT mixed with OVA, they found that sublingual administration in mice triggered immune changes throughout the body. There were significant IgA and IgG antibody responses both in serum and in mucosal tissues including the lungs and genital tract. The CT/OVA mixture also prompted the release of a mixture of cytokines mediating cell and antibody immunity and generated cytotoxic T cell responses peripherally and in the lungs. But for this robust response the use of the whole cholera toxin was important. When less toxic CTB was linked to OVA and delivered sublingually it induced immunological tolerance.

These researchers see the sublingual route of mucosal vaccination as promising. Compared to ingested antigen there is little degradation so much less antigen is needed. Compared to nasal administration there is less risk of neural toxicity. And as opposed to injection it is less invasive; no device is required to deliver the antigen and there's no issue of needle phobia.

Carlos Guzman of GBF-German Research Center for Biotechnology talked about new derivatives of another mucosal adjuvant, macrophage-activating lipopeptide of 2 kilodaltons (MALP-2). MALP-2 is a TLR ligand that binds the heterodimer TLR2/6 on macrophages. MALP-2 also promotes the activation and maturation of DCs and his group has recently demonstrated that it can directly stimulate B cells.

Guzman reported on a structure-function analysis aimed at dissecting the role of the fatty acids and peptide components of this molecule, as well as the impact of its stereochemistry. The studies revealed that the chiral center and fatty acid acylation were critical for MALP-2 function as an adjuvant. The peptide was a different story—when Guzman substituted polyethyleneglycol (PEG) for the peptide, the resulting molecule was as good an adjuvant as MALP-2 by a number of immunological criteria, but was more stable and soluble and could be manufactured at lower cost.

HIV prevention in a pill?

Can drugs that combat herpes virus help reduce HIV transmission?

by Catherine Zandonella

If ongoing clinical trials pan out, it's possible that one day people could be cutting their risk of HIV infection simply by popping a couple of pills per day. The pills are cheap, safe, and have been on the market for years. The catch? These drugs don't target HIV, they fight off herpes.

Simply quelling genital herpes could be enough to substantially reduce an individual's risk of acquiring and transmitting HIV. Researchers have long known that sexually transmitted infections (STIs) play a facilitative role in HIV transmission. Now nearly a dozen clinical trials are investigating whether drugs that suppress herpes simplex virus-2 (HSV-2), the causative agent of genital herpes, can reduce HIV transmission.

Preventive approaches that aim to modify behavior are yielding only modest gains against HIV transmission, so many researchers are now exploring such innovative biological preventive measures—earlier this year a clinical trial in South Africa found that male circumcision could reduce the risk of HIV acquisition (see *Cutting HIV transmission*, *IAVI Report* **9**, 3, 2005).

Prevention strategies that focus on biology rather than behavior, or a combination of the two, may provide greater gains in prevention than a behavioral approach alone. "Aside from individual behavior change, we don't really have ways to prevent HIV transmission," says Anna Wald, an epidemiologist at the University of Washington School of Public Health and Community Medicine, Seattle. "That is the starting point of why we are looking at herpes."

Aside from individual behavior change, we don't really have ways to prevent HIV transmission. That is the starting point of why we are looking at herpes

Anna Wald

But before researchers can go distributing drugs against herpes to HIV at-risk populations they need to demonstrate that these medications can reduce HIV transmission in real world settings, says Jairam Lingappa, medical director of one of the studies organized by the University of Washington. "While epidemiologic studies show a relationship between HIV and genital herpes," he says, "we don't yet have a clear demonstration of the public health benefit of using HSV-2 suppression."

Herpes is a lifelong infection and the virus cycles between latency and reactivation to cause clinical disease, producing painful ulcers at the genital mucosa. Numerous studies have found a strong association between genital ulcer diseases and increased HIV transmission (*Herpes* **11** Suppl. 1, 36A, 2004). HIV is rather inefficient at spreading via sexual intercourse, leaping from one person to another in perhaps as few as one time out of every 1000 sexual exposures under certain conditions. Genital sores facilitate transmission by disrupting the physical barrier of the skin and enabling HIV to much more easily enter the body. In addition, genital herpes causes inflammation that recruits dendritic cells (DCs) and activated $CD4^+$ T cells to the genital mucosa. There, DCs can entrap HIV particles and then traffic them to $CD4^+$ T cells at distant sites like the lymph nodes.

So controlling these ulcers should reduce HIV transmission. Yet two large trials in the early 1990s, one conducted in Mwanzaa, Tanzania and the other in Rakai, Uganda, yielded conflicting results. The trials involved treating curable STIs such as syphilis, gonorrhea, chlamydia, and trichomoniasis, but not genital herpes, a strategy that resulted in a 40% reduction in HIV-transmission rate in Tanzania but no reduction in Uganda. The differing results are thought to be due to population characteristics, notably that the Tanzania cohort exhibited more high-risk behavior than the cohort in Uganda, where the HIV epidemic was more mature. (*J. Infect Dis.* **191** Suppl. 1, S168, 2005). However, some researchers believe that another factor was the lack of herpes treatment, which is not curable and is the most widespread STI in sub-Saharan Africa. In some regions of Africa, HSV-2 seroprevalence is greater than 80% in men and women 35 and older. (*J. Infect. Dis.* **186** Suppl. 1, S3, 2002).

Vicious cycle

Individuals with genital herpes are at 3 times greater risk of acquiring HIV, according to a just published meta-analysis of 19 studies (*AIDS* **20**, 73, 2006) conducted by Esther Freeman and colleagues at the London School of Hygiene and Tropical Medicine (LSHTM). The increase in risk was roughly the same in both men and women in the general population.

What is more, the proportion of HIV transmission events due to HSV-2 could be on the rise, according to a presentation from Freeman at the July 2005 International Society for Sexually Transmitted Disease Research meeting in Amsterdam. "The population-attributable fraction of new HIV infections due to HSV-2 is increasing over time in African populations," she said.

This elevated risk of acquiring HIV may be greatest in the first few months following infection with HSV-2, when severe outbreaks are most common (*J. Infect. Dis.* **187**, 1513, 2003). "The severity of these first episodes appears to account for the ability of HIV to infect the individual," says Steven Reynolds, an infectious disease specialist now at the US National Institutes of Health working in Uganda.

Genital herpes heightens the risk not only of acquiring HIV but also of transmitting it to a sexual partner. HSV-2 replication, even if actual herpes sores are not present, can lead to more frequent and greater amounts of HIV shedding in the genital tract since HSV-2 regulatory proteins can interact with a regulatory region of the HIV genome to upregulate its replication. And acute episodes of genital herpes result in transient increases of HIV RNA levels in the plasma (*J. Acquir. Immune Defic. Syndr.* **35**, 435, 2004).

In addition, HSV-2 is one of the most common opportunistic infections and takes advantage of the immunocompromised status of HIV-infected persons to cause frequent and prolonged outbreaks of genital herpes. So the vicious cycle is complete: HIV-related immunosuppression causes more HSV-2 to be present in the genital tract, which in turn promotes more HIV replication.

These lines of evidence open up the exciting possibility that suppressing HSV-2 could reduce both the risk of acquiring HIV (acquisition) and the risk of transmitting it to a sexual partner (infectiousness).

> **The... fraction of new HIV infections due to HSV-2 is increasing over time in African populations**
>
> **Esther Freeman**

Herpes suppression on trial

To examine the public health benefit, a number of clinical trials are starting up to assess whether giving a drug to suppress HSV-2 can knock down HIV transmission (Table 1). These randomized, blinded, placebo-controlled trials will evaluate treatment with acyclovir, a proven anti-herpes drug efficient at suppressing HSV-2 activity, particularly clinical episodes.

Acyclovir is affordable, it has few side effects, and the virus rarely becomes resistant, even after years. The drug is typically used in two ways: as a long-term suppressive therapy and as an episodic treatment when herpes flares up. The suppressive regimen helps keep HSV-2 latent, reducing the frequency and severity of outbreaks. The episodic treatment shortens the duration of the outbreak, although only by about one day out of a six-day period. Often by the time people seek treatment the outbreak has been going on for several days. Because of this relatively small improvement, acyclovir is not a standard treatment for genital herpes under World Health Organization guidelines.

Yet some researchers, including Philippe Mayaud of the LSHTM, think episodic treatment could significantly reduce the amount of HIV shed. He is involved in three studies, one of which is looking at HIV transmission when acyclovir is given to women in Ghana and the Central African Republic who come to clinics seeking treatment for genital herpes. Women who consent to be in the study are HIV tested and offered acyclovir three times a day for five days or a placebo. Mayaud and his colleagues will take genital samples from all women to see if they shed less HSV-2 and HIV (the latter among HIV-infected women only) due to the acyclovir treatment; the HIV-uninfected women will be followed to see if acyclovir protects them from acquiring HIV. "If you control the HSV-2 shedding it may have longer lasting effect on HIV shedding than just the duration of the episodic treatment per se," says Mayaud.

Episodic versus suppressive therapy

Episodic treatment alone may not be enough, however. It does not control asymptomatic HSV-2 shedding, a situation that researchers now know is extremely common. "If you looked only at the symptomatic phase, you'd miss a lot of the shedding," says Lingappa. So Mayaud and other research teams are exploring suppressive therapy.

One such study is testing this concept in female bar- and hotel-workers in Tanzania. A suppressive regimen of acyclovir or a placebo is being given to 1000 HIV-infected and HIV-uninfected women in a trial led by Debby Watson-Jones at the LSHTM. The HIV-uninfected women are being monitored for seroconversion, while the HIV-infected women are being monitored to see if the suppressive acyclovir regimen decreases their HSV-2 shedding and consequently their HIV shedding. Together with colleagues from Burkina Faso and the University of Montpellier in France, Mayaud is also exploring how highly active antiretroviral therapy (HAART) may alter HSV-2's impact on HIV shedding. "All these shedding studies will be very important benchmarks for the effect of different interventions for HSV-2 or HIV," he says.

While shedding studies are important, what researchers really want to know is whether acyclovir can reduce HIV acquisition in people who have HSV-2. A large scale study to answer that question is being conducted by Wald and Connie Celum, also of the University of Washington. The study is following women in three African nations (South Africa, Zambia and Zimbabwe) and men who have sex with men (MSM) in the US and Peru to see if acyclovir gives them protection against acquiring HIV. The researchers will also be looking at how well the drug controls the occurrence and frequency of genital ulcers, whether the participants adhered to the regimen of two pills daily, and also the effect of acyclovir on HIV setpoint in HIV seroconverters. "The trial of 3200 women and men is over 80% enrolled with excellent retention and adherence, so we

Ongoing Trials of HSV-2 Suppression

Study PI	Population	Treatment	Regimen	Primary Outcome	Sponsor
Connie Celum and Anna Wald, University of Washington	1600 HIV-/HSV-2+ MSM in USA and Peru.1600 HIV-/HSV-2+ women in Zambia, Zimbabwe, and South Africa	Suppressive	Acyclovir 400 mg twice daily for 18 months	HIV infection	National Institutes of Health; University of Washington (Coordinating Center)
Connie Celum, Jai Lingappa, and Anna Wald, University of Washington	2800 discordant couples in which one partner is HIV+/HSV-2+ in South Africa, Kenya, Tanzania, Uganda, Rwanda, Zambia, Botswana	Suppressive	Acyclovir 400 mg twice daily for up to 24 months given to HIV+/HSV-2+ partner	HIV transmission to non-infected partner	Bill and Melinda Gates Foundation; University of Washington (Coordinating Center)
Anna Wald, University of Washington	60 HIV+/HSV-2+ MSM in USA	Suppressive	Valacyclovir 1.0 g daily for 8 weeks	HIV shedding	University of Washington; GlaxoSmithKline
Connie Celum, University of Washington	40 HIV+/HSV-2+ MSM and women in Peru	Suppressive	Valacyclovir 1.0 g daily for 8 weeks	Plasma and mucosal HIV levels	University of Washington; GlaxoSmithKline
Philippe Mayaud and Nicolas Nagot, LSHTM; and Philippe Van de Perre, University of Montpellier	150 HIV+/HSV2+ women not needing ARV; 60 on ARV in Burkina Faso	Suppressive	Valacyclovir 1.0 g daily for 3 months	HIV and HSV-2 shedding	French National Agency for Research on AIDS and Viral Hepatitis
Philippe Mayaud and Sinead Delany-Moretlwe, LSHTM; and Reproductive Health Research Unit, South Africa	300 HIV+/HSV-2+ women in Johannesburg, South Africa	Suppressive	Acyclovir 400 mg twice daily for 3 months	HIV and HSV-2 shedding	Wellcome Trust
Debby Watson-Jones, LSHTM	1000 female bar-workers (HIV+ and HIV-) in Mwanza & Shinyanga, Tanzania	Suppressive	Acyclovir 400 mg twice daily for 2 years	HIV seroconversion among HIV-; HIV/HSV-2 shedding in HIV+	Wellcome Trust
Philippe Mayaud and Helen Weiss, LSHTM; Laurent Belec, INSERM U430 Paris	Population: 500 women (HIV+ and HIV-) with genital ulcer disease in Ghana, Central African Republic	Episodic	Acyclovir 400 mg 3 times daily for 5 days	HIV and HSV-2 shedding; HIV/HSV-2 seroconversion	French National Agency for Research on AIDS and Viral Hepatitis
Sam Phiri and Philippe Mayaud, LSHTM; and University of North Carolina	500 men and women (HIV+ and HIV-) with genital ulcer disease in Lilongwe, Malawi	Episodic	Acyclovir 400 mg 3 times daily for 5 days	Ulcer healing and HIV/HSV-2 shedding; HIV seroconversion	Fogarty International Centre; UK Department for International Development
Gabriela Paz-Bailey, Centers for Disease Control and Prevention; David Lewis, National Institute for Communicable Diseases, South Africa	600 HIV+ men with genital ulcer disease in Johannesburg, South Africa	Episodic	Acyclovir 400 mg 3 times daily for 5 days	Ulcer healing, lesional HIV shedding; HIV seroconversion	Centers for Disease Control and Prevention; LSHTM
Francois-Xavier Mbopi-Keou, Fred Hutchinson Cancer Research Center	40 HIV+/HSV-2+ women in Cameroon	Suppressive	Acyclovir 400 mg twice daily for 8 weeks	Genital HIV shedding	Fred Hutchinson Cancer Research Center; Institute for the Development of Africa

Sources: Philippe Mayaud and Clinicaltrials.gov

Table 1. Ongoing trials to evaluate episodic or suppressive treatment of HSV-2 for the prevention of HIV acquisition or transmission. *The trial participants are either HIV-infected (HIV+) or HIV-noninfected (HIV-) and HSV-2-infected (HSV-2+) or HSV-2-noninfected (HSV-2-).* ***ARV****: antiretroviral;* ***MSM****: men who have sex with men;* ***P I****: Principle Investigator;* ***LSHTM****: London School of Hygiene and Tropical Medicine*

are optimistic that we will get an answer about the degree to which genital herpes increases HIV susceptibility," says Celum.

The buck stops here

Another important question is whether acyclovir can prevent HIV-infected individuals from passing the virus to a partner. The best way to answer this question is with a study of so-called "HIV discordant" couples, where one partner is infected with both HIV and HSV-2 and the other partner is not infected with HIV. Such a study is now starting up led by Celum, Wald, and Lingappa. The study will track nearly 3000 HIV discordant couples at twelve sites in seven African countries. The HIV-infected, HSV-2-infected partner will be given either acyclovir or a placebo to see if they are at reduced risk of passing HIV to their non-infected partner, in the context of couples'

counseling, bacterial STI treatment, and condom provision.

If acyclovir proves capable of reducing HIV transmission the trial results will benefit everyone—but none so much as the discordant couples themselves. HSV-2 is the biggest cause of genital ulcers in married couples, says Susan Allen, a professor at Emory University's Rollins School of Public Health and a pioneer in studying HIV-discordant couples. "When there is an HIV transmission event," says Allen, "85% of the time the virus is acquired from the spouse."

An exciting aspect of the study of herpes suppression among HIV-discordant couples is the implementation of strategies to promote couples' counseling and recruitment of HIV-discordant couples in Africa, says Celum. A counseling program developed collaboratively by Allen, Liverpool Voluntary Counseling and Testing, and staff at the US Centers for Disease Control and Prevention has been used at some study sites to help couples understand the risks they face and help them make choices about how to minimize that risk. "This time period [when one partner is infected and the other is not] is a critical window in which to implement a public health strategy to reduce transmission," says Lingappa. "If we can enhance the number of families that maintain one healthy parent or adult, that is one of the things we should promote."

While well designed, no study can answer every question. The couples study is meant to examine acyclovir's role in preventing HIV transmission to the HIV-uninfected partner, but it does not test whether a greater reduction in intra-couple transmission could result if both members of the couple took acyclovir, to prevent both the infected partner from transmitting HIV and the uninfected partner from acquiring it. By studying acquisition and infectiousness in two separate trials, the researchers run the risk of finding only weak associations in both. But Celum says the team carefully considered combining the trials and decided it would be better to separate the studies to determine the relative impact of acyclovir on acquisition and infectiousness.

A medicine for the masses?

If the trials are successful, what will that mean for the average person who is at risk of either acquiring the disease or, if they have it already, transmitting it to a partner? Mayaud hopes that if the episodic treatment proves successful acyclovir will be offered as a standard treatment for genital herpes when people visit a clinic.

Suppressive therapy—that is, a 400 mg pill of acyclovir twice a day for years—will be expensive and difficult to distribute in Africa. Valacyclovir, the newer form that can be taken just once a day, is not yet produced generically. A year's course of generic suppression therapy could cost as little as US$40 per year in Africa, but that is still prohibitive in most settings. Despite the costs, most researchers argue that if there is a remedy on the shelf that can be used to reduce HIV transmission it should be made available. "Until the day comes when an effective AIDS vaccine is developed," says Pat Fast, medical director at the International AIDS Vaccine Initiative, "researchers must try everything they can to stem the spread of HIV."

"If you ask me can you deal with a global epidemic by just giving someone a pill, my answer is no," says Wald, who adds that she hopes the trials will add impetus to developing an HSV-2 vaccine. "However, I feel strongly that if the results of this trial are positive, it will clearly be very important on an individual basis to use this for prevention of HIV acquisition or transmission."

Catherine Zandonella, MPH, is a freelance writer whose work has appeared in Nature *and* New Scientist.

> ***If we can enhance the number of families that maintain one healthy parent or adult, that is one of the things we should promote***
>
> ***Jairam Lingappa***

An Interview with

Barton Haynes

A new virtual Center

Bart Haynes, MD, has been interested in a wide spectrum of immunology during his research career, from autoimmunity—especially rheumatoid arthritis—to thymus biology and thymus transplantation as a curative treatment for DiGeorge syndrome, to HIV soon after it was identified as the causative agent of AIDS in the 1980s. He has long been affiliated with Duke University, North Carolina, having completed his residency training after gaining his medical degree at Baylor College of Medicine, Houston. Haynes began his research career under the mentorship of Sheldon Wolff and Anthony Fauci at the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), before returning to join the faculty at Duke in 1980. There he has held a number of senior positions, including chief of the Division of Rheumatology, Allergy, and Clinical Immunology (1987-1995), chair of the Department of Medicine (1995-2002), and is currently director of the Duke University Human Vaccine Institute. Haynes has also served as chair of the NIAID AIDS Vaccine Research Working Group that advises the NIH.

Haynes leads a team that in July was awarded the prestigious Center for HIV/AIDS Vaccine Immunology (CHAVI) grant from NIAID. CHAVI is a 'virtual consortium' that brings together researchers in a close collaborative effort that is hoped will speed the progress towards an AIDS vaccine by addressing key immunological questions in HIV infection, and design, develop, and test novel vaccine candidates. It will receive more than US$300 million over seven years. Haynes spoke recently to* IAVI Report *Editor Simon Noble about his vision for CHAVI.

What are your initial priorities with CHAVI, and what have you been able to achieve so far?

Our first priority is to establish a functional organization and I think we've made great strides so far. One of the driving forces of the Global HIV/AIDS Vaccine Enterprise, when Rick Klausner, Tony Fauci, Gary Nabel, myself, and others wrote the commentary proposing the concept in 2003 (*Science* **300**, 2036, 2003), was that we were all frustrated at the slow tempo of research at the time and the limited progress that had been made towards a practical vaccine. CHAVI is the NIH's component of the Vaccine Enterprise.

The overall goals of CHAVI are: to elucidate early viral and immunological events and host genetic factors associated with HIV-1 transmission, establishment of productive infection, and (partial) containment of virus replication; to determine correlates of SIV immune protection in nonhuman primates; to design, develop, and test novel immunogens and adjuvants that elicit persistent mucosal and/or systemic immune responses in humans and nonhuman primates; and to evaluate HIV vaccine candidates in early phase clinical trials. Our initial Scientific Leadership Group is Joe Sodroski and Norm Letvin from Harvard, George Shaw from University of Alabama at Birmingham (UAB) and Andrew McMichael from Oxford.

Now, there are two ways that you can set up a very large, complicated organization like CHAVI. The traditional way is to set up sequentially, one component at a time. When we learned that we had been awarded the CHAVI grant we decided to do everything in parallel to speed progress. So the first people I hired were an intellectual property (IP) person and a lawyer to incorporate the IP issues into the research plan that we had submitted in application. Then we immediately began writing clinical protocols based upon our research plan, and we started dealing with all of the related QA/QC issues of both clinical trial design and clinical sample assays. We immediately contracted with Family Health International for site management of our clinical programs. We also contracted with SCHARP and Frontier Sciences to develop our patient, sample, and central relational databases that can process not only the samples from the patients and their clinical data, but relate data coming from the host and virus genetic studies, and then all of the functional assays that are key to each patient and each time of blood draw. We should be functional and begin to acquire patient samples in the first quarter of '06.

We've had a series of CHAVI strategic planning sessions to discuss the possibility of mucosal and innate immunity studies, and also for laboratory test standard operating procedure (SOP) development. We've also written four clinical protocols to date on acute HIV infection patients and exposed uninfected subjects and we're in the process of getting the necessary approvals for implementation in early 2006. NIH staff have been wonderfully supportive in moving our clinical protocols through the review process.

It's been quite chaotic but I told everybody from the beginning that out of this controlled chaos will come both creativity and an accelerated way of working. At our organizational meeting in August there were 166 people there. I brought not only the scientists but also all the administrators from our scientific sites and from the NIH, and I told the administrators that they are going to be just as critical to CHAVI's success as the scientists.

The CHAVI RFA (request for applications) emphasized two scientific priorities identified by the Vaccine Enterprise: early immunologic and virologic events in HIV-infected/exposed people, and correlates of immune protection from SIV infection in nonhuman primate models. How do you plan to study early events?

We're going to look at the very earliest stages of acute HIV infection using a new pooling strategy of samples from STD clinics developed by Mike Cohen, Chris Pilcher, and Joe Eron and colleagues at University of North Carolina (UNC). The pooling strategy screens samples with RNA PCR to detect virus positive, antibody negative samples at the very early stages, hopefully even when virus load is still increasing. This is one of the strategies that differentiate CHAVI's programs, to find patients at the very earliest stages of HIV infection.

For the studies looking at the ontogeny of T and B cell responses with changes in viral load we'll need sufficient numbers of patients within the first two to four weeks of infection to really get an idea of what the transmission event is like, what the effects are on the host's innate and adaptive immune responses, and to map out what happens in those people that control the virus versus those that aren't able to initially control.

For this we need a series of collaborating sites that can establish this screening protocol to identify patients immediately after the transmission event, so Mike Cohen has begun to establish collaborations with investigators in Durban (Salim Karim) and Johannesburg (Helen Rees and Wendy Stevens) in South Africa, Lilongwe (Mike Cohen) and Blantyre (Taha Taha) in Malawi, Entebbe (Pontiano Kaleebu and Heiner Grosskurth) in Uganda, and Moshi (Saidi Kapiga, John Shao, John Crump and John Bartlett) in Tanzania, as well as in London (Sarah Fidler and Phillipa Easterbrook) and the US (UNC—Joe Eron, Chris Pilcher; Duke—Charles Hicks). These collaborations will provide the samples for the work that CHAVI investigators will perform We're collaborating with existing robust sites that are already set up to do this type of accrual work. As many as possible of the studies will be performed on site. We are also talking to other networks about the possibility of collaboration as well. The less CHAVI has to duplicate, the more time and resources we can spend on vaccine development.

We've also canvassed all of our collaborators and found that we have about 70 retrospective samples that are of the quality that we plan to collect prospectively. We're just beginning now to work on these, to set up assays, and to begin to work out all of the problems of data analysis, standardization, SOPs.

We're collaborating with IAVI on one of our first studies, CHAVI 002, on HIV exposed and uninfected individuals. A pilot group of patient samples will be tested by Andrew McMichael in Oxford, and will be simultaneously studied by Jill Gilmour in the London IAVI laboratory. IAVI is being a great help in developing the laboratory procedures standardization, because they have previous experience. It's a good example of how CHAVI is going to work, collaborate and synergize with folks who are doing things already that are helpful to the field, so that we don't waste either time or resources reinventing the wheel where it's not necessary.

How does CHAVI plan to investigate the correlates of immune protection in the SIV/macaque model?

In our original application this study was not expanded to the degree that we ultimately planned that it would be because of limitations in the original grant. So in the second year of the grant, Norm Letvin with multiple collaborators is now putting together an expanded program that will focus on two areas; one is to determine the correlates of protection in live attenuated SIV infection and the other is to study host-virus interactions in dual infections, and to study these in tandem. When live attenuated infection is protective, what are the immune correlates of that protection? And when superinfection occurs, what has the first infection done to the immune system to allow the second infection? That program is just being worked out, and so we'll have more details in a couple of months.

So you're pretty pleased with progress so far?

I'm thrilled with progress so far, because everyone has accepted this philosophy and has dealt marvelously with the controlled chaos that has come out of trying to do things differently. I think that's one of the things that CHAVI can contribute to the field, to figure out how to do things in a quicker, more effective

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way. I told everyone that the secret to success here is that everyone puts this organization on their back and when something comes across their desk that needs to be done for CHAVI, they stop what they're doing and get it off their desk, don't let it languish. Immediately move it. The constant challenge is going to be to maintain that momentum over time, but so far I'm pleased with the early stages of establishing a functional organization.

It's interesting that you identified IP as an initial priority. Has that been a bottleneck on progress in previous collaborations, the willingness and ability of people to share reagents and ideas?

Absolutely, the exchange of reagents and the whole issue of whether one can use certain strategies and what have you is always a traditional bottleneck. We already have a CHAVI materials transfer agreement that we can sign once and then enhance the flow of reagents among all of our investigators. Secondly, our IP manager and legal counsel together are currently canvassing all CHAVI investigators and creating an inventory of the existing CHAVI IP portfolio. Number three, we've begun educating all CHAVI investigators on how to protect future IP, so that we have control over it and can make it available to whoever needs it in order to optimally speed the development of a vaccine, should we be so fortunate to be in that position.

There are currently six Discovery Teams in CHAVI: immunology, vaccine design, adjuvant development, viral biology, structural biology, host genetics and genomics. New Discovery Teams will be set up after the first year—what kinds of subject areas do you see being included?

For year 02, we are considering new teams in mucosal immunity, innate immunity, and computational biology and mathematical modeling. This year, Ray Dolin at Harvard is working to design what our first clinical trials might be in year 03, and David Goldstein at Duke is working to expand the CHAVI host genetics programs. Finally, for year 03 we are also considering a mother-to-child transmission (MTCT) team.

What's the primary goal of the computational biology and mathematical modeling team?

It's three-fold. One is to have a team that can analyze the hugely complex data sets that will come out of our acute HIV infection and exposed uninfected observational studies. Secondly, to be able to model the human immune system with regard to observed responses and to predict how vaccines will interact with the immune system based upon responses seen in those individuals that can control the virus. And then third, to help us with vaccine design, particularly focused on viral genetics. The mathematical modeling group will assist Beatrice Hahn at UAB in viral genetics, and also will work with Joe Sodroski and Steve Harrison at Harvard in their structural analysis of the transmitted HIV envelope trimer.

We're currently in discussion with various groups with regard to who might participate. A group that's already on board that has been a tremendous help to CHAVI investigators is the Los Alamos Mathematical Biology and Computational Immunology team, led by Bette Korber and Tanmoy Bhattacharya. I've been working with Bette for over 15 years on immunogen design using computational biology techniques.

One of the outcomes of the CHAVI acute HIV infection observational studies will be a transmitted virus database of full-length sequences of the virus that gets transmitted across the bottleneck at mucosal sites. I think these kinds of sequence analyses are really the only way to design immunogens that can induce T-cell responses that have a chance of dealing with virus diversity, as well as resolving whether transmitted viruses are different to the viruses that evolve later in HIV infection.

To be able to model the human immune system with regard to observed responses and to predict how vaccines will interact with the immune system

If a MTCT team is added to CHAVI what will be its research priorities?

We believe that it's important to ultimately develop a vaccine that will be practical for children as well as adults. MTCT is quite complicated because there are three points of transmission: *in utero*, peripartum, and during breast feeding. So the first fundamental questions are: Is the biology of transmission at those three time points the same or different? Exactly what kind of vaccine would need to be given to a mother so that either she will be protected or, if she becomes infected, her child will be protected? A separate issue is developing childhood vaccines that would be effective, given the differences in a child's versus an adult's immune system.

In year 02 we'll start a strategic planning process to determine whether CHAVI has the resources to address MTCT, but personally I believe this is a very important area to consider. There are so many things that one could study. Whether it's MTCT, innate immunity, adaptive immunity, one of CHAVI's tasks is to decide what the key questions are in all of these areas. Yes, there are many interesting questions, but are they the most relevant questions for vaccine development?

Which vaccine vectors will CHAVI focus on and why?

One of the charges to CHAVI is to develop vectors that induce long-lasting, protective, mucosal immunity. During our strategic planning process we asked which vectors and inserts had the greatest promise for doing that, and initially we will focus on attenuated

recombinant VSV [vesicular stomatitis virus], chimeric adenovirus, and recombinant mycobacteria.

We'll have a team working on mucosal assay development and standardization, which we think is critical to collecting the high quality data needed in order to evaluate mucosal vaccines. We'll be combining mucosal and systemic immunogenicity studies for a vector analysis comparing this group of vectors containing the same insert. Then we will compare a concomitant group of inserts in the same vector, and determine which inserts best induce broad neutralizing-antibody and T-cell responses.

VSV can induce robust T- and B-cell responses, similar to adenovirus, and in some studies can induce mucosal as well as systemic responses. Also, VSV can be administered intranasally and at other mucosal sites, and we'll be evaluating these. Of course the problem with VSV has been the concern of neurotoxicity but we'll be working with those who have developed some new attenuated strains that may well be less neurotoxic, to evaluate them for their immunogenicity.

We're interested in mycobacteria because of the duration of responses that BCG can induce, because BCG and other mycobacteria have been administered orally for many years and are generally safe, and because millions of children get BCG soon after birth. I've had an NIH grant with Bill Jacobs and Norm Letvin for three years to develop a multivalent mycobacterial-vector vaccine incorporating HIV genes for a combined TB/HIV vaccine. That work will now be coordinated with CHAVI work.

What are the major practical and theoretical hurdles in studying/measuring mucosal immune responses?

The practical problems are getting samples in the first place, and then ensuring that they are informative and in sufficient volumes. Theoretically, the key is understanding what the infectious unit is—cell-associated or cell-free virus—the role of local versus systemic immunity in protection at mucosal sites, the first cell or cells infected at transmission, and the nature of the infecting HIV quasispecies. To name just a few.

There's been a drive for some years now to standardize reagents and assays, to enable comparison of results across experimental systems and research groups. Is that going to be a driving force within CHAVI?

There are folks who are already doing this for existing organizations. David Montefiori is doing this with regard to neutralizing antibodies at the HVTN, and so David is leading this effort for CHAVI. Rick Koup is already doing this with regard to T-cell assays for the VRC, and he is working with Clive Gray, Kent Weinhold, and Guido Ferrari at the HVTN, and they're all now also working with CHAVI. Steve Self at SCHARP is working with the Vaccine Enterprise, HVTN, and now CHAVI for database development and statistical support. So by picking key individuals who are already doing this for other key organizations, we hope to speed the harmonization of what CHAVI does and, again, not reinvent the wheel.

The CHAVI grant application required a detailed research plan. What is the process for expanding or changing the scope of that research plan?

We can go out and seek new collaborations and discovery teams, and other folks that think they have ideas or cohorts to offer can contact us. I think that the community needs to view CHAVI not as a funding agency but as a "company" charged with studying the correlates of protective immunity to HIV and developing new vectors for induction of long lasting mucosal immunity, to speed HIV vaccine development. There are many exciting things scientifically that can be done, but we have been tasked to focus on those things that we believe will speed vaccine development.

The community needs to view CHAVI... as a "company" charged with studying the correlates of protective immunity to HIV and developing new vectors

Will there be a mechanism to consider ideas from outside of the CHAVI researchers?

Yes, AIDS researchers can contact CHAVI with ideas using a two page letter of interest. CHAVI has a defined mechanism to vet new ideas for new discovery teams culminating in review by our CHAVI Executive Committee that is comprised of NIAID staff and our Scientific Advisory Board.

How much of the CHAVI funding, about $49 million/year, will be available as grants to researchers outside of CHAVI?

We don't yet know the status of our year 02 budget, so it's unknown at present. But any funds expended on new programs or investigators that were not in the original grant will be done in a manner such that the new investigators will become members of the CHAVI team, and work to move the "company's" work forward with the same research agreement and policies that initial CHAVI investigators signed on to.

How can young investigators be attracted to HIV research, and can CHAVI play a role?

Absolutely, CHAVI plans to bend over backwards to help the field, including helping to build enthusiasm and to help train the next generation of AIDS vaccine development researchers. A critical issue is how to bring young people into CHAVI teams and convince them that their work will benefit their careers. We are already working with Duke department chairs and promotions committees to educate them of the value of working in research teams at universities, and of recognizing the contributions of young investigators for the work they do in teams. We have to have young people come to this field to bring in the new ideas that we hope will help solve the AIDS vaccine problem.

Renewed promise

Annual AIDS vaccine meeting highlights recent data from clinical trials and lessons on recruitment and retention of volunteers

by Kristen Jill Kresge

Vaccine researchers and immunologists can be forgiven if, at times, they seem overwhelmed by the challenges that lie ahead. Working through the scientific difficulties inherent in developing a vaccine, the disappointing results from clinical trials, and the inconsistent data from animal models could make anyone a little skeptical, if not downright depressed. Seemingly, this despair has not gone unnoticed. "This field could use a little Elavil or Prozac," said Larry Corey of the HIV Vaccine Trials Network (HVTN) to scientists gathered at the AIDS Vaccine 2005 conference in Montreal in September. In conversation, several other researchers shared Corey's opinion about the level of frustration in the field.

But over the following days the hundreds of researchers who gathered were handed a remedy for their depression, albeit in small doses. Results from two Phase I studies with DNA-based vaccines showed promise and sparked a renewed interest in this strategy, illustrating that not all DNA candidates are created equal. Meanwhile Merck's lead candidate, MRKAd5, yielded promising results on many fronts, including its immunogenicity in people with pre-existing immunity to the adenovirus serotype 5 (Ad5) vector followed by encouraging news from the field's only ongoing Phase IIb trial. More preliminary research into new vaccine vectors also attracted attention at the meeting. Wyeth's manipulations of vesicular stomatitis virus (VSV) have rendered the viral vector nearly ready for testing in human volunteers and data from several animal models demonstrating the improved safety of this approach dominated the presentations.

There was also a wealth of information on recruiting and retaining volunteers from the clinical trials and vaccine preparatory studies that continue across sites spanning several continents. Researchers brought up some of the remaining ethical dilemmas involved with the design of clinical trials, including the need to evaluate AIDS vaccines in adolescent volunteers. Dealing with these issues will become essential as candidates advance and the field readies for large-scale efficacy trials.

When Corey addressed the delegates again at the closing ceremony, he encouraged the field to move forward with guarded optimism despite remaining obstacles. He predicted a fruitful year of research that will provide substantial data on vaccines that induce cell-mediated immunity. And if that translates into progress, then it could be even better than Prozac.

DNA down but not out

The Vaccine Research Center (VRC) at the US National Institutes of Health has tested a series of DNA vaccines in several Phase I clinical trials. In one of these trials, VRC 004, volunteers received a 4 plasmid DNA vaccination including *env* genes from subtypes A, B, C and a fused *gag/pol/nef* construct from subtype B. These volunteers were subsequently rolled over into the VRC 009 trial where they received a booster inoculation with a combination of 4 Ad5 recombinants that were previously tested alone in the VRC 006 trial (see *Research at the extremes, IAVI Report* **9**, 2, 2005).

Barney Graham of the VRC presented the impressive results of this rollover study in Montreal. All volunteers received a 10^{10} dose of Ad5 after receiving either 4 or 8 mg of DNA in the previous trial. Researchers observed an 11 to 21-fold rise in immune responses following the adenovirus boost, including a substantial increase in antibody response in volunteers with low Ad5 titers at the start of the VRC 009 trial. Graham also reported that the $CD8^+$ T-cell responses after boosting were comparable to those seen in long term nonprogressors. In volunteers with higher Ad5 antibody titers, the responses were dampened similarly to those reported from other studies.

The results of this study differ from those Merck reported with its DNA/Ad5 vaccine combination, which was abandoned because it offered no advantage over the MRKAd5 candidate alone (now in Phase IIb trial). Graham speculated that there are several possible reasons for this difference, including the addition of the *env* gene that was not included in Merck's DNA candidate as well as the design of the VRC 009 study, which as a rollover study had at least a two-year gap between the DNA prime vaccination and the Ad5 boost. "The long boosting interval probably does play a role in the improved immune response," Graham said at the meeting. He acknowledged that this represents an impractical vaccination schedule, but argued that these results warrant further research. "DNA is immunogenic and this data shows that it clearly primes."

The VRC is now running a Phase II clinical trial of this DNA/Ad5 combination vaccine to assess its immunogenicity with a more standard prime/boost schedule. The randomized, placebo-controlled study, HVTN 204, will enroll 480 volunteers at HVTN sites in North and South America, Africa, and the Caribbean. Volunteers will receive three injections with the DNA prime and a single Ad5 boost over a period of six months. This candidate is also being tested in a series of Phase I and II trials at sites in East Africa including Kigali, Rwanda, in cooperation with IAVI, at other African sites with the US Military Research Program, and sites in Haiti, Puerto Rico, Jamaica, Brazil, and the US with the HVTN (see *Vaccine Briefs*, this issue).

Results from another Phase I trial with a DNA candidate developed by Advanced BioScience Laboratories and tested at the University of Massachusetts Medical School were also presented at the meeting. The candidate includes a DNA prime containing a subtype C *gag* gene and *env* from five HIV subtypes followed by a homologous gp120 protein booster vaccination with an adjuvant called QS-21. The trial included two groups of volunteers that received a 1.2 mg dose of DNA administered either intradermally or intramuscularly, and a third group that received a 7.2 mg dose of DNA intramuscularly, followed in all arms by a 0.375 mg protein boost.

The trial was stopped prematurely upon advisement from a Data Safety Monitoring Board due to a case of cutaneous small-vesicle vasculitis that developed in a single volunteer in the high dose arm.

Other adverse events that were reported included grade 2 flu-like symptoms. But Jeff Kennedy, assistant professor of medicine at the University of Massachusetts, believes that further study is still merited. "We're not sure how concerned we are about this. In vaccine development sometimes reactogenicity isn't a bad thing," he says. Kennedy cited smallpox and yellow fever vaccines as clear examples of approved products that can have side-effects but were developed because of the human toll these diseases wrought.

The candidate induced robust immune responses as determined by interferon-γ (IFN-γ) ELISPOT even prior to the Ad5 boost, and high antibody titers were detected by ELISA at all doses after boosting, with 60-70% of volunteers mounting a substantial antibody response to HIV subtype B. Although promising, Kennedy invokes a baseball analogy in describing the results from this Phase I trial. "It's more like hitting a double with no outs than a walk-off home run. Now we have to figure out how to get these responses even higher." He predicts the future of this candidate is to test it in combination with other promising approaches, including an adenovirus vector.

Modifications in test of concept study

Robin Isaacs, executive director of HIV Vaccine Clinical Research at Merck, presented the rationale behind the company's decision to modify the Phase IIb or test of concept trial—which is testing the trivalent Ad5 vector expressing subtype B Gag, Pol, and Nef proteins—to include volunteers with high vector-specific antibody titers. The trial was originally designed to exclude any volunteers with Ad5 specific titers greater than 1:200, but after analysis of now completed studies with the trivalent vaccine researchers concluded that at higher doses there was less inhibition of immune responses in people with pre-existing immunity than expected.

At the $3x10^{10}$ particle dose of the trivalent vaccine, 69% of the 118 volunteers produced an immune response to at least two proteins in IFN-γ ELISPOT. When this data was broken down by the level of pre-existing immunity, 77% of the volunteers with titers less than or equal to 1:200 were classified as having the same response. This finding was consistent with other studies that illustrated a broader immune response than previously seen with the monovalent Gag Ad5 candidate. The trivalent vaccine even seems to be immunogenic in people with very high antibody titers, reaching beyond 1:1000.

"We have found that the trivalent vaccine consistently produces a detectable immune response in 60-70% of people and that the impact of Ad5 was less when we used it in people who had pre-existing immunity," says Isaacs. These results led the company to include an equal number of volunteers with high (>1:200) and low levels (≤1:200) of pre-existing antibody immunity to the viral vector, in essence doubling the enrollment in the Phase IIb trial by including an additional 1500 volunteers in the new cohort. The so-called Step study will expand enrollment across all currently active Merck and HVTN sites and may also begin recruiting volunteers at new sites in North America, South America, the Caribbean, and Australia. The primary and secondary endpoints of this ongoing trial are the prevention of infection and the ability to slow disease progression in volunteers that become infected during the course of the trial. Isaacs reported that approximately 1200 volunteers have received the Ad5 candidate so far in all clinical trials and that the only side-effects observed are injection site reactions and fever after initial dosing, especially in volunteers with low adenovirus-specific antibody titers. Results of this intermediate study are not expected until 2008.

> ***If we continue to find that people with high Ad5 antibodies have a good response to the vaccine then it may well make the vaccine useful for a larger number of people***
>
> **Robin Isaacs**

"If we continue to find that people with high Ad5 antibodies have a good response to the vaccine then it may well make the vaccine useful for a larger number of people," says Isaacs. In the meantime Merck continues to look at other serotypes, including Ad6, in order to broaden the immunogenicity of the vaccine and find what he refers to as the "ultimate" adenovirus vector.

VSV vector nears testing in human trials

John Rose of Yale University has been developing VSV as a vaccine vector for many years and this negative-stranded RNA virus, which is a natural pathogen of cattle, has proven highly effective. Just a single intranasal inoculation with a VSV vector expressing hemagglutinin from influenza virus elicits impressive neutralizing antibody titers in mice. Proteins from measles virus or the Severe Acute Respiratory Syndrome (SARS) coronavirus expressed in VSV have also provided impressive results in animal models.

In 2001 Rose reported that an AIDS vaccine candidate based on attenuated VSV vectors was able to protect seven rhesus macaques from disease up to 14 months after a SHIV89.6P virus challenge (*Cell* **106**, 539, 2001). All but one of the control animals in this experiment progressed to AIDS in an average of 148 days. These results caught the attention of researchers at Wyeth who began working on developing this approach into a candidate that could be evaluated in human clinical trials.

Although VSV does not cause serious disease in humans it is a replication competent vector and so determining its neurovirulence profile was a priority for Wyeth. Researchers injected wild-type and recombinant forms of the virus directly into the brains of macaques. All animals that received the wild-type VSV developed necrotizing meningoencephalitis, a slowly progressing viral infection of the

brain, while some also showed hemorrhaging and inflammation around the blood vessels. The recombinant forms induced less severe necrosis, but it was clear that further attenuation of the vector was necessary.

Wyeth researchers tried various genetic manipulations, including shuffling the nucleocapsid gene further downstream in the viral sequence in order to decrease expression, truncating or deleting the transmembrane glycoprotein ectodomain that is responsible for viral attachment, and introducing mutations into the matrix protein to reduce cytopathic effects. These strategies were tested alone and in combination in several animal models, including mice and ferrets.

David Clarke of Wyeth reported in Montreal that the newly engineered VSV vectors were all "extremely attenuated". In ferrets these attenuated vectors induced only minor lesions upon direct injection into the brain. Intranasal administration led to a further reduction of lesions and caused only minor swelling, but as Clarke notes there is bound to be some inflammation when any agent is delivered directly into the brain.

The researchers then identified two of these attenuated vectors as lead candidates—referred to as N4CT9 and N4CT1—and tested them in both mice and young macaques. With the wild-type VSV they observed mortality in mice with only a 10^2 particle dose; however with the N4CT1 it took a 10^7 particle dose to induce mortality. Kevin Wright, also of Wyeth, presented immunogenicity data of these rationally designed variants at the conference. Mice that received a 10^7 particle intramuscular injection both as a prime and a boost generated cellular responses after the boost equivalent to a single inoculation with wild-type. The anti-Gag responses and the antibody responses as measured by ELISA were even greater with the attenuated viruses than wild type VSV.

The immunogenicity data in monkeys parallels that in mice, according to Stephen Udem, vice president of viral vaccine research at Wyeth. Udem also presented on these vectors at the meeting and waxed poetic over their potential, even going as far as calling it the "best vector on Earth," at least in animals. He remarked that although he is continually surprised by the mutation capacity of HIV, he is also repeatedly impressed by the potency of VSV as a vector. And the genetically modified vector, N4CT9, is now being manufactured in preparation for Phase I clinical trials.

"We've done almost everything we can do in animals," says Wright. "Now we have to see how it works in humans. Let's hope it bears fruit."

Wyeth has had several meetings with the US Food and Drug Administration (FDA) who will be responsible for approving the Investigational New Drug application that the company is now filing. The approach is "well regarded" by the FDA according to Udem, who predicts that the vaccine will be in human trials within a year. "The N4CT9 vector produced less injury than vaccines we've been using for hundreds of years, like smallpox," says Udem. "It's a remarkably effective agent." The trial will evaluate an intramuscular rather than intranasal administration because it induces more robust immune responses.

Learning from experience

As more vaccine candidates enter or progress in clinical trials it will be necessary to recruit more volunteers, especially women, and optimizing the design of vaccine trials was another important focus of the conference. Several presentations in Montreal centered on some of the difficulties experienced or progress made during enrollment for ongoing trials or cohort studies.

Stephen Mawa of Makerere University and the Walter Reed Project in Kampala, Uganda gave a presentation on recruiting for a Phase I trial (VRC 009) in 2004 with the VRC's DNA candidate. The trial site staff conducted more than 20 informative seminars for both community leaders and the general public—each attended by as many as 100 people—and used newspaper and radio advertisements to recruit potential volunteers. In total the site interacted with more than 4000 people and although several hundred women were screened, only a few were enrolled. Despite these extensive outreach efforts the majority of volunteers reported hearing about the study from friends and Mawa emphasized the need to engage in continuous advocacy and education since "word of mouth" is always likely to be an important recruitment tool.

Sanjay Mehendale of the National AIDS Research Institute in Pune, India, where the country's first AIDS vaccine trial began earlier this year, presented more encouraging news on recruiting women for this Phase I vaccine trial. Preparations included sensitizing the trial staff about the need to recruit women, conducting meetings with local women's groups, as well as training the staff on gender-related issues that could affect participation. The IAVI-sponsored Phase I study enrolled 11 male and 9 female volunteers to receive either the low or medium-level doses of the adeno-associated virus based vaccine called tgAAC09.

But to get just nine female volunteers enrolled the site staff had to screen five times that number, indicating that significant screening resources will be necessary if investigators are to recruit a balanced number of women in larger Phase II or III clinical trials in this country.

In preparation for these trials Project San Francisco (PSF), a project founded in Kigali, Rwanda in 1986, has been working extensively with discordant male-female African couples where one partner is HIV infected and the other is not. The PSF sites have been successful at bringing women into clinics for HIV testing and possible enrollment in a vaccine trial and are the world's largest cohorts of HIV serodiscordant couples.

More than 20,000 couples have been screened at one of PSF's sites in Kigali over the past three years, 950 of whom have been identified as serodiscordant, according to a presentation by Erin Shutes who works at this site. The retention rates for these cohorts hover around 90% through one year, offering a unique opportunity for investigators to reach potential female volunteers for vaccine trials. The Kigali cohort is now enrolling volunteers for a Phase I vaccine trial.

Beyond the strategies for recruiting female volunteers, researchers within the AIDS vaccine community are also starting to grapple with the possibility of evaluating promising candidates in adolescent volunteers. The inclusion of adolescents in AIDS vaccine trials bring up completely new ethical questions and will be an important issue for debate and discussion in the coming years. The Montreal meeting provided merely the background for what will be a thorny issue in vaccine research, but as Glenda Gray of the University of Witswatersand reminded researchers, "HIV prevalence among adolescents in South Africa is horrific and excluding them from efficacy trials is a big mistake."

Research Briefs

Attacking HIV from inside and out

Two recent reports suggest that the use of the same antiviral taken orally or applied vaginally as a microbicide can prevent immunodeficiency virus infection in a rhesus macaque model. While either treatment was able to lower the frequency of infection, the authors conclude that effective prevention may require a multi-pronged approach with ARVs introduced by both routes, perhaps in combination with a partially-effective vaccine.

In the first report, John Moore of Weill Medical College of Cornell University and Ronald Veazey of Tulane National Primate Research Center and their colleagues explored the use of three different types of compounds in a vaginal microbicide: BMS-378806 (produced by Bristol-Myers Squibb), a gp120-binding small molecule inhibitor that prevents viral attachment to CD4 and CCR5 receptors; another small molecule called CMPD167 (produced by Merck), which clogs the gp120 binding site on CCR5; and C52L, a fusion-inhibiting peptide similar in sequence to the drug T20 (or enfuvirtide).

The researchers used a high virus dose vaginal challenge model of infection in macaques using SHIV-162P3, a chimeric virus with an HIV envelope and an SIV core. All nine control animals became infected. In contrast, all three ARVs delivered individually or in combinations were able to lower infection rate—only 11 out of 51 animals receiving some combination of the drugs became infected. The data also suggested that the two drugs could be applied hours before and still provide some protection (*Nature* **438**, 99, 2005).

In a separate report, the same research groups used this macaque model to test whether oral delivery of CMPD167 could prevent vaginal infection by SHIV. The drug was given either twice a day for 4 days prior to viral challenge, or 10 days after challenge, or both before and after. While the orally-delivered drug appears to prevent infection, the results were less clear cut than when the same drug was used in a microbicide.

Only the treatment group where animals received no drug before infection and twice daily doses for 10 days after infection had a statistically-significant reduction in infection rate. But taking into account all animals that received the drug post-challenge (including those which received it pre-challenge as well), the reduction in infection rate was much more significant. Only 10 out of 20 animals receiving drug after SHIV challenge became infected, as opposed to 16 out of 18 of control animals (*Nature Medicine* **11**, 1293, 2005).

Whether either route of delivery will provide protection from infection in humans isn't clear. But as a step towards taking one of these strategies to the clinic, Bristol-Myers Squibb and Merck recently announced they had granted the International Partnership for Microbicides a royalty-free license to use BMS-378806 and CMPD167 or closely-related compounds in microbicide trials in developing countries.

A new way to snuff the viral fuse

A crucial step in HIV's replication cycle is when the virus fuses with its target cells. Inhibiting the process of viral fusion has become a promising approach for HIV therapeutics and is the mode of action of T20 (or enfuvirtide), a drug that works by binding the membrane fusion-promoting gp41 protein. Now John Shiver's team at Merck and their colleagues report that a monoclonal antibody that binds the same gp41 region as T20 is able to inhibit viral fusion of diverse HIV-1 clinical isolates, suggesting a novel strategy for eliciting broadly neutralizing antibodies in a preventive vaccine (*Proc. Natl Acad. Sci. USA* **102**, 14759, 2005).

T20 blocks HIV fusion by binding the heptad repeat 1 (HR1) region of gp41. After HIV attaches to a cell through gp120, the HR1 region plays an important structural role in a series of rapid and dramatic shufflings of protein domains that culminates in fusion of the viral and cellular membranes. In particular, T20 stops a transition of HR1 from a so-called prehairpin structure to a bundle of six alpha-helices. Shiver and his team began their search by selecting antibodies from a phage library of single chain antigen-binding Fv antibody regions derived from normal human B cells, using two peptides designed to mimic the HR1 structure in the prehairpin as their target antigen. This process identified 100 different candidates.

The researchers then tested these in an HIV fusion assay. The report describes full characterization of one promising Fv, named 5H, which inhibited fusion in a dose dependent manner. They demonstrated that this antigen-binding region maintained its ability to inhibit fusion even when it was converted to a full IgG molecule. They also confirmed through mutational, biochemical, and structural analysis that the antibody binding site on gp41 overlapped with highly conserved amino acids in the HR1 region.

This monoclonal antibody was able to neutralize diverse HIV isolates, 9 out of 19 viruses tested, including examples from subtypes B, C, and F. However, compared to broadly neutralizing antibodies which have been isolated from HIV-infected individuals—IgG1b12 and 2F5—D5 was at least 10 times less potent and neutralized fewer HIV isolates. And T20, which binds the same region and presumably acts by the same mechanism, was an extremely potent inhibitor of all 19 strains. Shiver's group is now trying to select more potent variants of the antibody by using *in vitro* antibody evolution.

The team also evaluated the ability of their prehairpin mimics to elicit HIV neutralizing antibodies when injected into rhesus macaques. Unfortunately this has repeatedly resulted in high-titer non-neutralizing antibodies. But the researchers point out that by studying the binding properties of D5 they may be able to design immunogens that elicit more powerful neutralizing antibodies to the same epitope.

Research Briefs

Hooking up to stimulate immunity

Using drugs that activate Toll-like receptors (TLRs) to stimulate adaptive immunity is a hot topic among vaccine researchers (see *Toll bridge to immunity*, page 1). Now Robert Seder of the Vaccine Research Center at the NIH and his colleagues report that such TLR agonists can help stimulate both cellular and humoral immunity against HIV Gag protein in nonhuman primates—and they see dramatic improvement in the cellular response when the drug and protein are chemically linked.

Experiments in mice and cultured human cells have shown that TLRs influence adaptive immunity by activating dendritic cells (DCs), which present antigen to T cells and release factors that stimulate T cell differentiation and expansion. But whether TLRs can do the same in humans or nonhuman primates hasn't been clear. Seder's group has now tested the ability of three different TLR agonists to elicit T cell responses to HIV Gag protein in Indian rhesus macaques (*Proc. Natl Acad. Sci. USA* **102**, 15190, 2005).

The animals received an injection of Gag alone or mixed with a solution containing a different agonist against either TLR7/8 (3M-012), TLR8 (3M-002), or TLR9 (CpG oligodeoxynucleotide) four times at four week intervals. In addition, one group received an injection of Gag crosslinked to TLR7/8 agonist with ultraviolet light. At different time points, Seder's team performed ELISPOT assays to determine the frequency of IL-2 and IFN-γ producing T cells. Animals receiving the TLR7/8 or TLR9 agonists had significantly higher levels of IL-2^+ and IFN-γ^+ T cells compared to macaques receiving only Gag protein. In contrast, the TLR8 agonist had little effect. But linking the TLR7/8 agonist to the protein had a dramatic effect. Six weeks after the final injection, IL-2^+ and IFN-γ^+ T cells were six-fold higher for the protein-linked agonist compared to the same agonist delivered with the protein as separate molecules.

The researchers then used nine-color flow cyotmetry to more finely characterize the Gag-specific memory T cell response. They measured production of IL-2, which is important in sustaining memory, and IFN-γ and TNF-α, which mediate effector function. Here the effect of the conjugation was just as striking. The highest average frequency of cytokine-producing $CD4^+$ and $CD8^+$ T cells was elicited by the protein-linked agonist. The quality of the response was also significantly altered. For the TLR7/8 or TLR 9 agonist in solution, about 40% of $CD4^+$ T cells were producing only IFN-γ and less than 25% were producing all three cytokines. But in response to the protein-TLR7/8 agonist conjugate, 40% of $CD4^+$ T cells were producing all three cytokines and about 25% were producing TNF-α and IFN-γ.

There was an even stronger contrast in the results for $CD8^+$ memory T cells. On average, the unlinked TLR agonists had no effect or barely increased the frequency of cytokine-producing $CD8^+$ T cells over the protein alone, while the linked agonist had a significant effect. Flow cytometry demonstrated that about 25% of these cells were producing all three cytokines, while 40% produced TNF-α and IFN-γ. With the exception of the TLR8 agonist, all the drugs elicited high titer antibodies against Gag.

The researchers demonstrated that this four injection protocol with the Gag-linked TLR agonist produced a T cell response comparable to replication-defective adenovirus expressing Gag, suggesting it could be used instead of or in conjunction with such viral vectors as a prime or boost. This team is now repeating their experiment with the TLR7/8 agonist linked to SIV Gag in order to test whether this vaccination protocol by itself can protect animals against challenge by SIV.

For whom the B cell tolls

The role of Toll-like receptor (TLR) activation in generating a humoral immune response goes beyond their ability to stimulate dendritic cells (DCs), concludes a new report by Chandrashekhar Pasare and Ruslan Medzhitov of Yale University. They have shown in mice that direct activation of TLRs on B cells is necessary for robust production of some classes of antigen-specific antibodies (*Nature* **438**, 364, 2005).

This work is part of a growing body of evidence supporting multiple roles for TLRs in generating adaptive immunity, making them of great interest to immunologists and vaccinologists (see *Toll bridge to immunity*, page 1). For example, when the gene for MyD88—a signaling adaptor protein crucial to the function of many TLRs—is knocked out in mice, the ability of these animals to mount strong antigen-specific antibody responses is severely compromised. This makes sense given the role of TLRs in stimulating DC maturation and activating T helper cells. However it wasn't clear if TLRs on B cells also played an important role.

To get at this question, the researchers used mouse genetics and cell transplants to test the effect of TLR signaling on B cells specifically. They started with mice with a defect in the immunoglobulin μ chain gene, which renders the rodents deficient in B cells. These animals were immunized with human serum albumin (HSA) and lipopolysaccharide (LPS), a ligand for TLR4, to generate HSA-specific memory T helper cells. After 90 days the animals received an infusion of purified naïve B cells from wild-type mice and were immunized again with either HSA alone or HSA with LPS. While memory T helper cell responses were comparable in both groups, the HSA-specific IgG1 response dropped by about half if LPS was not included in the immunization, suggesting TLR signaling on B cells boosted the response.

Indeed, when the researchers transferred B cells from mice with MyD88 or TLR4 knockouts into B cell deficient mice and then immunized with HSA and LPS, levels of HSA-specific IgM and IgG were produced at no greater than 25 per cent of levels produced by wild-type B cells. The defect appeared to be very specific to the production of certain Ig classes. The production of IgE antibodies was not significantly impaired in the MyD88 knockout B cells and there was no difference in the homing or survival between the MyD88 and TLR4 knockout B cells and wild-type.

Research Briefs *written by Philip Cohen*

Vaccine Briefs

Merck's HPV vaccine shines in Phase III trials

A vaccine to protect women from infection with human papilloma virus (HPV)—which causes cervical and anal cancer, as well as genital warts—was found 100% effective at preventing high-grade cervical pre-cancers as well as non-invasive cervical cancers associated with the strains of the virus that are contained in the vaccine. This is the first report from a large-scale efficacy trial with Merck's HPV vaccine, known as Gardasil.

This Phase III trial (FUTURE II) enrolled 12,167 women aged 16-26 at 90 sites in Brazil, Colombia, Denmark, Finland, Iceland, Mexico, Norway, Peru, Poland, Singapore, Sweden, the UK, and the US. Women in the trial received three injections of Gardasil, which is a virus-like particle vaccine containing the L1 nucleocapsid protein of 4 HPV strains delivered with an aluminum adjuvant. Two of the strains (16 and 18) are responsible for over 70% of cervical cancer cases worldwide, while the other two strains (6 and 11) cause more than 90% of genital warts, a benign manifestation of genital HPV infection.

A secondary endpoint analysis showed that one immunization with the vaccine reduced the risk of developing high-grade pre-cancer and non-invasive cervical cancer due to infection with strains 16 and 18 by 97%. This result provides a more realistic example of how the vaccine may be used since people often do not return for all three courses of a vaccine. There was only a single observed case of pre-cancerous cervical lesions in the vaccine group compared to 36 in those who received placebo. The sustainability of the immune response is yet to be determined as the participants in this trial were followed only for an average of two years. These results were presented at the annual meeting of the Infectious Disease Society of America that took place recently in San Francisco.

Merck submitted an application to the US Food and Drug Administration for approval and licensure to market and sell the first cervical cancer vaccine based on the results of this study and other efficacy trials in more than 25,000 women and men in 33 countries. The company expects to receive a license early next year. Merck has also formed a joint venture with Sanofi pasteur to license and market the vaccine in Europe and the application was recently submitted to the European Medicines Agency.

There are still ongoing studies to evaluate Gardasil's ability to prevent the incidence of anal cancer in men who have sex with men and as a way of creating herd immunity to further reduce the disease burden in women, but the company has not reported any results yet in male volunteers.

"Where the vaccine is really needed is in developing countries," says Jessica Kahn of the Cincinnati Children's Hospital. "It could have a tremendous impact there on mortality rates." Cervical cancer is one of the leading cancers among women and worldwide it causes more than 290,000 mortalities annually. The vast majority of these deaths occur in developing countries because there are few screening programs to provide women with regular Pap tests. HPV is one of the most common sexually-transmitted infections among sexually active individuals and a 100% effective vaccine promises to greatly reduce the associated disease burden.

Another HPV vaccine developed by GlaxoSmithKline Biologicals in Rixsensart, Belgium is also in Phase III clinical trials. This candidate, known as Cervarix, is also a virus-like particle vaccine but it only contains L1 proteins from HPV strains 16 and 18. Cervarix also utilizes a proprietary adjuvant known as AS04. The company is currently conducting five efficacy trials in 28,000 women worldwide but no results have yet been reported. In a Phase II study the vaccine was found to be 100% effective at preventing persistent HPV infection in women that received three injections of the vaccine. This candidate is expected to be submitted to the European regulatory authorities for licensure in the first half of 2006.

IAVI Report

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IAVI is a scientific organization founded in 1996 whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. IAVI focuses on four key areas: Accelerating scientific progress; education and advocacy; ensuring vaccine access and creating a more supportive environment for industrial involvement in HIV vaccine development.

IAVI (www.iavi.org) is a global not-for-profit organization working to accelerate the development of a vaccine to prevent HIV infection and AIDS. Founded in 1996 and operational in 23 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI also works to assure that a vaccine will be accessible to everyone who needs it. IAVI's financial and in-kind supporters include the Bill & Melinda Gates Foundation, the Rockefeller Foundation, the Starr Foundation; the Governments of Canada, Denmark, the European Union, Ireland, the Netherlands, Norway, Sweden, the United Kingdom, the United States and the Basque Country; multilateral organizations such as the World Bank; corporate donors including BD (Becton, Dickinson & Co.), Continental Airlines, DHL and Pfizer; leading AIDS charities such as Crusaid, Deutsche AIDS Stiftung, and the Until There's A Cure Foundation; and other private donors such as the Haas Charitable Trusts. For more information, see www.iavi.org.

Vaccine Briefs

Clinical trials yield promising results from two adenovirus-based vaccines

Enrollment in Merck's ongoing Phase IIb "test of concept" trial with the trivalent adenovirus serotype 5 (Ad5)-based vaccine, MRKAd5, was recently expanded to include double the number of volunteers originally planned at sites in North America, South America, Australia, and the Caribbean. The 1500 additional volunteers will include individuals with Ad5 vector-specific antibody titers greater than 1:200 who were originally screened out of the trial (see *Renewed promise,* page 18).

Merck's decision to include volunteers with high levels of pre-existing immunity to the Ad5 vector was based on the results of now completed studies that showed MRKAd5, when given at a high dose of $3x10^{10}$ viral particles, can elicit strong immune responses even in individuals with substantial antibody titers. Robin Isaacs of Merck says that the vaccine seems to still be immunogenic in volunteers with antibody titers over 1:1000. The trivalent vaccine expresses Gag, Pol, and Nef proteins of subtype B HIV and is being evaluated in the Step study, a collaboration between Merck, the HIV Vaccine Trials Network (HVTN), and the National Institutes of Allergy and Infectious Diseases. This trial started in January of this year and final results are expected in 2008.

Another Ad5-based vaccine candidate developed at the Vaccine Research Center (VRC) is now in Phase II testing in partnership with the HVTN (see *Renewed promise,* page 18). This trial uses a 4-plasmid DNA prime vaccination comprised of a fused *gag/pol/nef* construct from subtype B, the primary viral strain found in Europe and North America, and HIV *env* genes from subtypes A, B, and C, which are the subtypes most common in Africa and parts of Asia. This is followed by a boost vaccination with an Ad5 recombinant containing *gag*, *pol*, *nef*, and *env* genes. This study, HVTN 204, seeks to enroll 480 volunteers at 13 HVTN sites in North and South America, Africa, and the Caribbean to determine safety and immunogenicity.

The VRC has seen an improved response when the DNA and Ad5 are administered in a prime/boost protocol and results from a Phase I trial indicate that the approach produced a robust cellular and antibody response. In the Phase I study there was a 26 month interval between receipt of the prime and boost, but the Phase II trial will evaluate a more typical vaccination schedule. Volunteers that are randomly selected to receive the DNA/Ad5 candidate will receive three injections of the DNA and a single Ad5 boost over a period of six months. Half of the trial participants will be enrolled at HVTN sites in the Americas as well as in Haiti and Jamaica, while the other half will be at sites in South Africa and Botswana.

This vaccine is the first developed at the VRC to move into the second stage of clinical testing. "Clearly what's different about this candidate is that it is responding to the global epidemic," says Gary Nabel, director of the VRC. "It's important to evaluate a vaccine that could have a broad response." The US-based company Vical is manufacturing the DNA portion of the vaccine and the adenovirus vector was developed by the VRC in collaboration with GenVec. Researchers at the VRC also hope to get around the problem of pre-existing immunity to the Ad5 vector by using a higher dose.

This candidate will also be evaluated further in a series of Phase I and II clinical trials in Kenya and Rwanda in cooperation with IAVI and at sites in Uganda, Kenya, and Tanzania in partnership with the US Military HIV Research Program, pending regulatory approvals in these countries. Nabel says these partnerships will allow the VRC to learn more about the immune response to the vaccine approach in diverse groups of volunteers. "Each of these organizations has some special strength that they bring to the research and I'm really excited and pleased that they have taken the considerable effort to harmonize their trial plans," he adds.

US Senators introduce bill to accelerate AIDS vaccine research

US senators John Kerry of Massachusetts and Richard Lugar of Indiana introduced legislation in Congress recently that calls for increased funding to accelerate the research and development of vaccines for AIDS, tuberculosis, and malaria, as well as other infectious diseases. The proposal, called the "Vaccines for the New Millennium Act of 2005", highlights several ways that both the US government and private industry can develop a comprehensive strategy for bringing new and important vaccines to the people in greatest need.

The bill calls for an increase in the number of public-private partnerships as one strategy for achieving this objective, and mentions in particular IAVI, the Malaria Vaccine Initiative, and the Global TB Drug Facility as examples of these partnerships. Other strategies mentioned include exploring improved regulatory procedures or economic incentives—such as tax credits—for private companies to increase their involvement in the research and development of vaccines that target diseases primarily affecting developing countries. According to the bill only 10% of the world's research and development capacity is focused on diseases that affect 90% of the global population. Other incentives for industry participation suggested in the bill are Advance Market Commitments (see *If you build it, they will pay*, *IAVI Report* **9**, 3, 2005), by which governments, foundations or other players in the global health community ensure a market through agreeing to buy vaccines for developing countries at guaranteed prices.

Within the legislation, senators Kerry and Lugar refer to immunization as a "cheap, reliable, and effective" way to have a profound impact on global health throughout the world. The co-authors cite several examples of this including the eradication of smallpox, the elimination of polio in many areas of the world, and vaccines for diseases like measles and tetanus that have drastically reduced rates of childhood mortality. The proposed legislation is yet to receive approval by the US government.

Vaccine Briefs *written by Kristen Jill Kresge*