It makes good sense that a key component of the nascent Global HIV/AIDS Vaccine Enterprise scientific strategic plan is to expand the standardization of immunoassays. Some of the most fruitful collaborations so far within vaccine trial sponsor organizations, as well as between them, have been built on the widespread adoption of a standardized assay to measure HIV specific cellular immune responses in vaccinees.

Everyone agrees that such assay standardization is a requirement to speed progress toward an effective AIDS vaccine. But some immunologists question whether the current gold standard will suffice. It’s a debate that continues because the assay field is still trying to get a firm grasp on underlying mechanisms.

The grand idea of forging a global partnership to accelerate the development of an effective AIDS vaccine took a step closer to reality with the publication of a scientific strategic plan identifying scientific roadblocks currently impeding progress. The plan from the Global HIV/AIDS Vaccine Enterprise, described as “an alliance of independent entities,” calls for a near doubling of worldwide investment in vaccine research and the coordination of an unprecedented network of researchers and labs between which reagents, data, and intellectual property will freely flow.

The plan was announced in January by the Enterprise in the Public Library of Science journal *PLoS Medicine*, after more than a year of work and input from more than 140 participants from 17 countries, including representatives from many of the key players in AIDS vaccine research and development: the Bill & Melinda Gates Foundation, the International AIDS Vaccine Initiative (IAVI), the National Agency for Research on AIDS (ANRS) of France, the US National Institutes of Health (NIH), the United Nations Joint Programme on HIV/AIDS (UNAIDS), the World Health Organization and the Wellcome Trust.

The document was greeted with endorsements and tough questions over the many remaining details of the plan that have yet to be resolved. Whether the Enterprise reaches these aspirations, experts say, will depend on how the plan is implemented and whether Enterprise members, outside scientists, funders, and other stakeholders rise to the many challenges that lie ahead.

The Enterprise began as the brainchild of the Bill & Melinda Gates Foundation, which was looking for ways to further accelerate AIDS vaccine research and development. They solicited input from a broad range of vaccine experts, including members of the Foundation, the
A hot topic at the meeting was the expansion of this tool kit, with 17 colors now available. Researchers have dubbed the new “multi-flavored” cytometry the “Baskin-Robbins” technique.

immunological principles, and determine just what constitutes a good indicator of an effective immune response against HIV— that is, the elusive immune correlates of protection.

For that reason, assay development remains very much a research endeavor. The field is exploring improved technology to visualize different aspects of the immune system and developing new, more powerful techniques to explore the incredible diversity of cells engaged in the immune response and their range of functions. These goals are part of what drew HIV researchers to the first Measurement of Antigen-Specific Immune Responses (MASIR) conference which took place in January in Courmayeur, Italy. The meeting attracted over 30 speakers and 134 participants from all over the world, and from multiple disciplines and arenas of immunobiology.

Participants included scientists, clinicians and technologists from academia and industry with research interests running from cancer immunology to viral pathogenesis and disease. The goals of the attendees also were diverse. Some were eager to view immunity at higher resolution in order to develop simple, powerful tests to diagnose disease and guide treatment. Others see the new technological approaches as a way to extend their knowledge about the mechanism of immunity. The cross-disciplinary forum allowed disparate investigators to compare and contrast approaches and conclusions.

Much of the research presented at the meeting aimed to clarify the role of CD4+ and CD8+ T cells in different aspects of immune responses to different pathogens or diseases. These cells are stimulated when they encounter antigens in the form of proteins broken down into peptides. The peptide antigens are presented to the T cells after they are taken up by antigen presenting cells and folded into a protein complex on the cell surface known as the major histocompatibility complex (MHC). This MHC-peptide complex is recognized by the T-cell receptor (TCR), another protein complex on the surface of CD4+ and CD8+ T cells (Figure 1).

Part of the challenge in deciphering immunity is the amazing complexity of T cells. Their surfaces can express a variety of markers that can be used to distinguish between different T cell populations such as CD8+ effector T cells, which react quickly to their target antigen, and CD8+ memory T cells, which are held in reserve, off-duty, in the thymus until the antigen they recognize makes a reappearance after an infection has been cleared. T cells are also capable of many different functions in response to antigen or other signals, ranging from the secretion of cytokines and chemokines that recruit other immune cells, to directly lysing infected cells. In addition, remarkable diversity comes from the TCRs, which are produced from genes assembled from genetic cassettes with different binding sites for MHC-peptide. To visualize some of that complexity, immunologists have relied on many standard molecular biology techniques such as monitoring gene regulation of T cells in response to different stages of infection and sequencing TCR genes. This work has benefited from advances in gene expression analysis (microarray) and sequencing which allow the rapid analysis of thousands of genes.

Another key technique is flow cytometry which can define the identity of cells, probe their function, and even separate cell populations for further analysis. In flow cytometry, cell surface proteins, or factors cells produce, are tagged with antibodies linked to different fluorescent color labels (Figure 1). Laser light is used to detect and quantify these signals or to sort cells into different populations for further analysis.

Only four of these fluorochromes have traditionally been available. But a hot topic at the meeting was the expansion of this tool kit, with 17 colors now available. Researchers have dubbed the new “multi-flavored” cytometry the “Baskin-Robbins” technique, after an American ice cream chain boasting 31 varieties. This advance is, of course, dependent on ingenious chemistry for new fluorochromes, but is also a testament to the plethora of markers that have been defined to characterize the T-cell type or function.

Some research presented at the MASIR meeting was geared toward finding novel proteins or genes that can be used as accurate indicators of the state of T cell activity. René van Lier from the Academic Medical Center in Amsterdam reported on his use of the cytomegalovirus (CMV) infection model to track down one such promising marker called IL-7Rα (CD127), a receptor which binds the cytokine interleukin (IL)-7. IL-7 is associated with maintenance and development of T-cell populations, especially naïve and memory subsets.

CMV, a herpesvirus, is ideally suited for studying virus-specific cell-mediated immune responses in healthy volunteers. The majority of adults over 40 years of age have been infected with CMV, regardless of socioeconomic or geographical location, and primary CMV infection is generally mild or asymptomatic. Normally, circulating virus cannot be detected and the virus remains latent, but CMV can reactivate if the host immune system
Figure 1. Probing the response of the immune system to HIV or SIV. HIV or SIV particles are taken up by antigen presenting cells (APCs) and their proteins processed into peptide antigens or epitopes. The peptides are transported to the APC surface bound in the major histocompatibility complex (MHC). The MHC-peptide complex is recognized by a T cell receptor (TCR). Immunologists use antibodies linked to fluorochromes to tag cells that produce certain factors (IFN-γ and IL-2 antibodies shown) or identify cells that possess particular cell surface markers (not shown).
is compromised by another virus infection such as HIV or through immunosuppressive drug therapy in preparation for a tissue transplant. When the virus is reactivated, CMV-specific $CD8^+$ T cells mediate effective immunity.

To better understand why those responses are successful, van Lier used a combination of techniques to study the cellular genes recruited during the T-cell response in the maintenance of CMV latency. First he used flow cytometry to separate different subtypes of T cells, including effectors and memory cells. Next, he went on a genetic fishing expedition, using gene chip arrays representing all known human genes to compare which ones responded to a new CMV infection versus an infection transitioning to a dormant state. This complicated analysis revealed that production or expression of the IL-7Rα protein dropped in effector T cells during primary CMV infection when virus is actively replicating and virus load is high. In contrast, after the immune system had suppressed CMV viremia, the effector T cells expressed increased levels of IL-7Rα. Van Lier proposed there was an inverse relationship between IL-7Rα and virus load of CMV during the infection.

The utility of IL-7 receptors as an indicator of effector function doesn’t seem limited to CMV. In separate presentations using flow cytometry to explore cellular immune responses in HIV and tuberculosis patients, Brigitte Autran, Université Paris VI Pierre et Marie Curie, Paris, and Dirk Busch, Universität München, Munich, independently found that disappearance of IL-7 receptor from the T cell surface indicated active $CD8^+$ T-cell effectors directed against the respective pathogens. Similar findings were recently reported by Rolf Zinkernagel and colleagues at the Institute of Experimental Immunology in Zurich, Switzerland (Eur. J. Immunol. 35, 738, 2005).

One reason the hunt for such new markers of immune function is heating up is the sense that the standard method for detection of immunological responses against cancer, bacterial or viral antigens doesn’t give the whole picture. Clinicians have relied on the detection of interferon (IFN) γ which is released by antigen-specific T cells when they recognize their target antigen. In most laboratories the IFNγ ELISPOT assay, which simply measures the number of T cells secreting IFNγ after binding to their cognate antigen peptides, is still the method of choice for screening immune responses, especially in developing countries. This technique is simple, quantifiable, robust, and relatively inexpensive.

However in her MASIR talk Sarah Rowland-Jones of Oxford University, UK, and MRC laboratories, Gambia, called into question the utility of IFNγ as a measure of the immune response in HIV infection. $CD8^+$ T cells are thought to play a major role in control of HIV replication, but Rowland-Jones contended that neither the high numbers of circulating HIV-specific $CD8^+$ T cells nor the magnitude of IFNγ responses necessarily correlated with viral load or clinical outcome.

Work presented by Guiseppe Pantaleo, CHUV, Lausanne, suggested that IFNγ measurements may be more useful if combined with detection of other markers of T cell function. His laboratory investigated the levels of IL-2 and IFNγ produced by both $CD4^+$ and $CD8^+$ T cell populations in conditions of acute (in Tetanus toxoid vaccinees), chronic (CMV, Epstein Barr virus [EBV], and herpes simplex virus [HSV]) or chronic persistent (HIV) immune responses. In general the production of IFNγ alone by antigen-specific T cells indicated the presence of high antigen load. Conversely, in conditions of low antigen load, an IL-2/IFNγ double secreting profile was observed. In acute CMV infection, for instance, he found a high frequency of $CD8^+$ T cells only producing IFNγ. After one year of infection after the virus entered its latent phase the frequency of IL-2/IFNγ producing T cells greatly increased. Also, HIV-infected individuals whose virus is being suppressed with HAART were found to produce a dual functional IL-2/IFNγ response compared to those off therapy with higher viral counts. Pantaleo also presented data from a single patient who controlled virus replication for 3 years without HAART. The patient demonstrated the IL-2/IFNγ double response profile against the HIV-Gag protein compared with an IFNγ only response they displayed during primary infection. Pantaleo suggested that more multifunctional $CD4^+$ and $CD8^+$ T-cell responses are associated with control of virus replication and that IFNγ responses alone are associated with “lack of control.”

Richard Koup and Michael Betts from the Vaccine Research Center (VRC) in Maryland took up the theme of multifunctionality in their talk. They took advantage of the full power of 17-color flow cytometry to define different classes of T cells based on their surface proteins and investigate several functions of these T cells simultaneously in HIV-infected individuals. This included analysis of CD107a, a marker of cell lysis function by cytotoxic T lymphocytes, and production of factors that modulate immune function including IFNγ, TNFα IL-2 and MIP1β.

This study focused on a cohort of 79 HIV-infected patients. Some patients were termed progressors, meaning the virus had already succeeded in damaging their immune system so that they had a low level of circulating
CD4⁺ T cells. Functional analysis of T cells from these individuals demonstrated a high frequency of CD107a, IFNγ and MIP1β expression but low IL-2 and TNFα. Another group of the patients were chosen because they possessed the human leukocyte antigen HLA-B57, which has been associated with maintenance of CD4⁺ T-cell counts and slow or non-progression to AIDS. In his analysis, Betts found that these individuals produced a high frequency of all the markers including IL-2 and TNFα.

Koup also used multicolored flow cytometry to analyze the functional immune responses elicited by immunization with a DNA vaccine composed of four plasmids (encoding envelopes from 3 different clades and a gag/pol/nef gene fusion) in comparison with those from HIV-nonprogressor individuals. Koup’s data showed that DNA immunization elicited CD4⁺ T cells that produced an IFNγ/IL-2 functional profile, whereas, the CD8⁺ T cells produced IFNγ alone. Koup noted that this particular DNA vaccine immunization elicited an intermediate T cell functional profile that lay between HIV-progressor and nonprogressor populations. His team will be using multi-paramater flow cytometry to analyze the immune response to different HIV immunization regimens so as to compare with the HIV non-progressor functional profiles.

Krishna Komanduri of the MD Anderson Cancer Center says the VRC group’s and Pantaleo’s presentations demonstrate the wide range of approaches investigators are taking to characterize immune responses. “It was interesting on one end of the spectrum that some individuals are pursuing ‘extreme’ flow cytometry to characterize the extent of variation within functional T cells while others… have become reductionist, simplifying relevant T-cell function to IL-2/IFNγ secretion capability.”

Speakers at MASIR also considered the TCR characteristics that govern T cell interaction with the MHC-peptide complex. There is potential for a diversity of TCR rearrangements to recognize the same MHC-peptide complex. TCRs consist of linked α and β proteins, encoded by genes which are assembled by the imprecise joining of the large number of variable (V), diversity (D, for β-chain only), and junctional (J) elements and the addition of extra nucleotides at the junctions that contributes to an enormous potential diversity. After a selection process which takes place in the thymus, mature T cells enter the periphery and form the preimmune repertoire available for recruitment in immune responses.

TCR repertoires in the periphery are selected by different antigens and have been found to vary widely in complexity. TCRs can also be grouped by the presence of specific motifs in the complementarity-determining regions (CDRs) at the TCR VDJ regions at the β-loci (TCR VB), a region which contacts and recognizes peptide in the context of MHC.

Mark Wills of Cambridge University, UK used the CMV model to study the successful immune response exhibited by long term healthy CMV carriers. For example, Wills described two individuals who shared the HLA-B7 MHC allele and showed similar amino acid sequence motifs within different TCR VB families present. He noted that within three weeks CD8⁺ T-cell responses became highly focused and dominated by a few heavily expanded TCRs.

At a later presentation, David Price of the VRC examined the antigen-specific TCR repertoire at the very fine level of CDR structure that represent the contact points of the TCR with peptide. Sequencing analysis revealed that protection against CMV was conferred by T cell populations with diverse CDRs. Together, this pair of presentations suggested that getting a complete picture of the TCR repertoire warrants examination of both the αβ backbone and CDR. “No longer can we assume that a response to a given [peptide] epitope is an immunologic unit, but rather contains a diverse set of responses,” says Koup.

However, CMV is a DNA virus that demonstrates little sequence variation when compared to genetically unstable RNA retro-viruses such as HIV or SIV. One of the significant stumbling blocks towards the development of an efficacious vaccine for HIV is that the virus can rapidly mutate leading to extensive sequence variation during an infection, which can disrupt recognition by T cells and result in lack of immune control.

In the non-human primate model, for example, single amino acid mutations within specific immunodominant peptide epitopes of the SIV Tat protein occur within as little as four weeks post infection and abrogate TCR recognition. But it’s intriguing that other immunodominant epitopes of the SIV Gag protein remain largely intact and thus remain targets for specific T-cell responses for a prolonged period. Investigators have attributed that difference to the relatively high ability of Tat to withstand structural change and yet maintain function.

Price and Daniel Douek of the VRC explored the role that the structure of the TCR plays in this process of viral escape. In rhesus macaque monkeys infected with a defined SIV isolate and possessing the same MHC background, the investigators sequenced 3,416 TCR sequences from CD8⁺ T-cell populations that recognize immunodominant peptides within the SIV Gag and Tat proteins.

It was known that the Gag-CM9 peptide mutates slowly after infection and exhibits escape late in infection. Price and Douek found that this relatively stable SIV peptide was recognized by a population of T cells similar to those that control CMV infection. They saw a reduced clonal diversity of TCR β-gene usage at 12 weeks and the Gag-CM9 specific T-cell CDRs were highly diverse, showing limited consensus motifs among the macaques analyzed. In contrast the Tat-TL8 peptide, which is known to exhibit rapid sequence variation consistent with escape shortly after infection, remained significantly more polyclonal at the β-loci after 12 weeks of infection. But examination of the precise molecular structural motifs within the T cell CDRs of the Tat-TL8-specific T cells revealed they were restricted in all 12 macaques studied.

Douek speculated that in contrast to the Gag-CM9 epitope, the change in the structure of the Tat-TL8 peptide would be significantly affected by amino acid variation, thereby altering the molecular structure of the peptide and subsequent recognition by the restricted CDR motif. It was concluded that diversity within the Gag-CM9 CDR motif confers CD8⁺ T-cell populations with promiscuous recognition properties such that they can tolerate a greater degree of sequence variation while maintaining effector function.

The MASIR conference highlighted one of the issues currently addressed by major HIV research groups, the ongoing hunt for relevant markers of effective immunity. While there is still much work to be done, one emerging lesson from all this research is that subtle intricacies of the immune response against HIV may be crucial and that the quality of an immune response is at least every bit as important as its magnitude.

Mario Roederer of the VRC says MASIR represented an important opportunity to talk about these issues with a very broad range of researchers. “I think the highlight of the meeting was the interactions among people. It was incredibly friendly and [there was] much discussion after every talk and poster.”

Adrian Mc Dermott, PhD, is Pre-Clinical Lab Manager for IAVI’s Research and Development team.
NIH and IAVI, resulting in a 2003 policy paper in the journal *Science* (300, 2036, 2003). That paper argued that a more systematic and efficient way to feed more promising vaccine candidates into the development pipeline was to forge a global “enterprise” to attack the problem, defined as “a high-quality collaborative research system that goes well beyond the high-quality but separate research projects that we have today.”

While collaborative efforts between independent labs are already underway, the hope is that the Enterprise will be a galvanizing force to encourage new collaborations and strengthen existing ones. However, the Enterprise is not intended to encompass all AIDS vaccine research, act as a managing bureaucracy, nor serve as a funding agency. Instead, its coordinating committee will help to shape and evaluate goals—the first of which was to draft the strategic plan—and establish a secretariat to provide logistical support and facilitate collaborations.

In its global collaborative vision, the Enterprise is often compared to the Human Genome Project, a network of public labs that divided the task of sequencing the three billion or so DNA bases in our chromosomes. The important lesson, according to Jose Esparza, senior advisor, HIV/AIDS at the Gates Foundation, is to think big. “People now realize that no individual institution can do this. Unless we think bigger and better we are not going to develop an effective vaccine in the near future.”

While the genome project provides a rough analogy, it is dwarfed by the ambitions of the Enterprise. For starters, even at current levels of funding AIDS vaccine research will spend in the next five years more than the US$3 billion price tag of the public genome sequencing. And while the elucidation of our genetic sequence was a technical tour de force, essentially no breakthroughs in scientific understanding were required and progress was easy to measure. The way forward for AIDS vaccine design is much less clear.

It’s not surprising then that while the strategic plan of the Enterprise acknowledges that many practical and organizational problems complicate AIDS vaccine development, they pinpoint the major barriers as scientific. So job number one the Enterprise set for itself was to prioritize the scientific issues to be tackled.

The *PLoS Medicine* paper provides this scientific strategic plan, identifying four major gaps in current knowledge that pose obstacles to vaccine development and would benefit from a comprehensive, coordinated research plan of attack.

**With a free-for-all, it’s difficult to compare results from different labs. You have two researchers with different assays, different read-outs and they can’t tell whose experiment is working better.**

Dennis Burton

The first area concerns questions about recently-transmitted viruses. A 2004 study (*Science*, 303, 2019, 2004) has suggested, for instance, that sexually-transmitted viral strains are not typical, but may be unusually sensitive to antibody neutralization *in vitro*. The aim is to establish a panel of virus isolates that better represents these recently-transmitted viruses, which are by definition the ones a preventative vaccine must fight, so learning more about this first line of viral invaders is crucial.

The next missing link identified by the plan are the elusive immune factors that account for protection in some animal models of the disease, particularly infection of rhesus macaque monkeys with simian immunodeficiency virus (SIV) or hybrid simian/human immunodeficiency viruses (SHIVs). When animals are vaccinated with live attenuated viruses they attain a very impressive level of immune protection against later virus challenge. Safety concerns prohibit this type of approach to protection being attempted in humans but it will be very instructive for researchers to understand what constitutes an effective immunity against viruses closely related to HIV in a model system.

Of course knowing how macaques fight the virus will be of little use if vaccinologists can’t find a way to elicit an effective response in humans. This leads to the last two areas that the Enterprise highlights as in dire need of illumination: strategies to induce antibodies that will neutralize genetically-diverse strains of the virus, and identifying vaccine candidates that elicit robust cellular immunity. Both arms of immunity have been targeted by past or existing vaccine candidates but have so far yielded disappointing results in clinical trials.

The plan doesn’t include any novel insights to crack these barriers but argues that its vision for a new level of global collaboration and coordination should help answer the questions more rapidly. For example, studies of newly-transmitted viruses would benefit from rapid and frequent communication between epidemiologists and clinicians who identify recently-infected people and laboratory researchers probing viral genetics and immune responses. Similarly, solving the mystery of protection in animal models would be sped along by coupling facilities with the resources to study large numbers of animals to generate statistically significant data and laboratories specializing in sophisticated immunological analysis. The plan proposes that consortia be formed to focus on these issues that, in turn, would be supported by a new infrastructure of labs providing a common stock of reagents and standardized assays across all efforts.

Dennis Burton of the Scripps Institute says this synchronization would immensely benefit the entire field. “With a free-for-all, it’s difficult to compare results from different labs,” he says. “You have two researchers with dif-

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**DECEMBER 2004-MARCH 2005**

**PLoS Medicine**

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**Science**
different assays, different read-outs and they can’t tell whose experiment is working better.” In contrast, central labs could guarantee that all researchers have access to the same panels of virus isolates to characterize antibodies or provide validated assays to assess immune responses in clinical trials. As a result, all the research efforts become part of a larger, consistent data set, giving every experiment more bang for the buck.

Many of the plan’s key scientific issues echo what AIDS vaccine researchers have been advocating for years and calls for steps that have already been incorporated within some ongoing research efforts. So it represents the current consensus of the field. But the Enterprise also represents a new commitment by many of those groups to strive for a higher level of coordination. The hope is that protocols could be broadly standardized, allowing wasteful overlap between projects to be eliminated and pushing researchers to study important areas that have formerly been neglected because they were too difficult for individual groups to tackle on their own.

“More resources can make a huge difference,” says Gary Nabel, director of the Vaccine Research Center (VRC) at the NIH. “There are experiments that never get done and things that aren’t attempted when resources are limited.” As an example, he cites hunting through crystallization conditions for HIV proteins. “This is extremely dull work,” he says. “There’s a practical limit to how much of it anyone can do day after day.” That’s why the VRC and IAVI’s Neutralizing Antibody Consortium are among the groups that have invested in untiring—but expensive—robots that can analyze as many conditions in one day as labs were formerly able to test in months.

Further downstream from the research laboratory, the plan describes a need to enlarge vaccine manufacturing capacity and for training more clinical professionals to test vaccines in human populations, particularly in developing countries where the toll of the virus is the greatest but clinical expertise lags far behind.

The plan was endorsed immediately by US senator Richard Lugar, chairman of the US Senate Foreign Relations committee, in a Washington Post editorial he co-authored with Patty Stonesifer, president of the Bill &

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**Figure 1.** The scientific strategic plan of the Enterprise identifies six key challenges to the development of an effective AIDS vaccine, and proposes the formation of consortia or centers to further accelerate vaccine research.

Adapted with permission from a figure created by the Bill & Melinda Gates Foundation
Melinda Gates Foundation. Bill Gates and Bono, U2 singer and co-founder of the advocacy group DATA (Debt, AIDS, Trade, Africa), used the opinion page of the UK newspaper The Daily Telegraph to express their hopes that leaders of developed nations would fund the activities of the Enterprise as part of a four-step plan to prioritize international development over the next year.

The Enterprise also seems to be part of a wider zeitgeist in which combating AIDS is on many politician’s lips. The Group of Eight (G8) leading industrialized nations endorsed the goals of the Enterprise as far back as June 2004. In the United Kingdom, Prime Minister Tony Blair has vowed to use Britain’s presidency of both the G8 and the European Union this year to focus attention on African poverty and health issues, including HIV. British Chancellor of the Exchequer Gordon Brown has even gone as far as proposing a mechanism for raising $50 billion in development funding with an International Finance Facility that would sell bonds on the global capital market. And at the recent annual meeting of the World Economic Forum, French President Jacques Chirac proposed his own revenue mechanism: new taxes on international financial transactions, jet and shipping fuel, and air plane travel tickets to fund the fight against AIDS.

How much a financial infusion this will ultimately mean for the Enterprise or AIDS vaccine research in general isn’t clear. But the Enterprise strategic plan calls for boosting the yearly investment in vaccine research from the less than $700 million currently spent to $1.2 billion.

Most of that new money has yet to materialize. The NIH has announced it will fund a Center for HIV/AIDS Vaccine Immunology (CHAVI) with a first year grant of $14.4 million, growing to $49 million in subsequent years, to address some of the obstacles. The Gates Foundation has recently issued a Request For Proposals in which consortia or centers can apply for $360 million over the next five years to support vaccine research targeted at priorities laid out in the Enterprise scientific plan. “The proof is in what happens next,” says Anthony Fauci, head of the NIH’s National Institute of Allergy and Infectious Disease. “What new commitments people make, what new funders come in, will decide whether this is real or not.”

However, the budget of the NIH, which next year will include $607 million of the world’s AIDS vaccine research funds, is set to grow only 0.5% next year and less than 2% annually until at least 2009.

While funding is crucial, Esparza says it isn’t surprising that it lags behind at this early point in the Enterprise’s development. “We need to do our homework and provide potential donors with the detail they need to see,” he says. That homework includes a comprehensive business document that will outline exactly where that new money is needed and how each dollar could help build a lab, say, or support a clinic. It will also elaborate the costs of not developing a vaccine and the financial rewards of curbing the epidemic. The Gates Foundation and IAVI are now collaborating to produce a comprehensive report.

“People strongly support the conceptual framework of more resources, more priority for an AIDS vaccine,” says Seth Berkley, president and CEO of IAVI. “The critical thing is that this is implemented in the right way,” he says. “The epidemic is global and so should be the response. We need to bring in the best researchers and companies in the world to work on this problem.”

Indeed, with the plan now published, the discussion and debate seems to have shifted to the best way to implement the plan, motivate those scientists and avoid repeating the mistakes of the past. In an accompanying editorial in PLoS Medicine, for example, a trio of editors, Virginia Barbour, Barbara Cohen and Gavin Yamey, pointed out the current plan contained no timelines, which they argued make it unlikely to light a fire under the vaccine community. Without a set of defined milestones they say “it will be impossible to define success and failure, review progress, and assure internal and external accountability.” However, even on this simple point of timelines a wide range of opinion can be found. Some vaccine experts think setting a clear list of objectives with timelines is critical for the Enterprise’s success, while others argue the devastation caused by the HIV pandemic is itself a sufficient motivator and that imposing a schedule on research progress is artificial.

This small disagreement demonstrates the greatest challenge the Enterprise faces—keeping far flung and independently-minded scientists and clinicians working together smoothly and focused on the same goal. Talk to a few members of the Enterprise and the challenge inevitably emerges in a simple phrase: everyone likes the idea of coordination, nobody likes to be coordinated. That will be all the more challenging since the Enterprise aims to link together a massive scientific network without a controlling central authority.

But for now, this loose structure is ideal
because there are so many possibilities to be explored, says Jerry Sadoff, president of the Aeras Global TB Vaccine Foundation and co-chair of the Enterprise’s vaccine manufacturing working group. “Early on you want a very egalitarian and open process. You don’t want to strangle anything that’s good,” he says. “But as the candidate number goes lower, the process needs to be more authoritarian, where you have to make tough decisions about what to bring forward and under these circumstances experience rules.” He doesn’t expect that process to break the Enterprise coalition, but he says it’s natural to expect some tensions to develop.

Another natural tension in the vaccine field is between academic and industrial partners. Nonetheless, Burton thinks the AIDS vaccine field could learn from industry’s focus on quickly identifying promising candidates and turning them into usable products. “Pure research projects are great ways to do science, but they are not enough for the HIV vaccine field,” he says. “You need a marriage between innovation and an industry-like vaccine effort to push things forward.” Klaus Cichutek at the Paul Ehrlich-Institut, the German Federal Agency for Sera and Vaccines, thinks another key area where the Enterprise could apply this corporate philosophy is in bridging the gap between basic researchers with promising preclinical vaccine candidates and clinical trials. Making the transition from bench to bedside requires quickly identifying promising candidates and clinical training program. Similar programs in the past have often been plagued by brain drain as workers take advantage of their new skills and seek better paying jobs in other countries. This seems like a particular risk for developing countries, with the right support, could use their baseline physiological measurements in the local population that will be extremely valuable in any future clinical research.

Even if the Enterprise achieves internal harmony, it will still face challenges, argues David Ho of the Aaron Diamond AIDS Research Center in New York City. He points out that the apparent strengths of the proposed Enterprise—its huge community of researchers and their coordination—could be its Achilles heel if it encourages a type of “group think” that stifles creative approaches. “We are still at a point in AIDS vaccine research where what we don’t know is greater than what we do know,” he says. “Therefore the conventional wisdom may not be correct. You need to keep fresh ideas percolating.” For AIDS vaccine research to succeed, he argues, it will be important that researchers outside the Enterprise are listened to and, just as crucially, funded. The plan seems to acknowledge this danger. “Small science’ should not be replaced with ‘big science.’ Both approaches must be taken,” it states.

Mitchell Warren from the AIDS Vaccine Advocacy Coalition believes that for the Enterprise to have global legitimacy, it will also need steady outside input from non-scientists such as experts in policy, public health and community leaders. “The challenge is that the Enterprise isn’t an elected body and you can’t give everyone with an interest a seat at the table,” he says. “But people will live with that if they can see who’s doing what, who’s funding it and how they ensure that the ball keeps moving forward.” The plan does propose annual stakeholders forums as one way to keep the Enterprise attuned to these outside voices.

As the Enterprise takes shape it is bound to face many challenges and questions. But Esparza says the greatest will always remain HIV itself. “We have been predicting a vaccine within ten years for the last twenty years, because HIV has proven to be more complex than we ever thought,” he says. “The scientific community now realizes that working collaboratively is the only chance we have. This realization made the Enterprise possible.”
Aches and Pains

Learning lessons from the influenza vaccine shortage

by Sheri Fink

As the US supply of influenza vaccine see-sawed from shortage to surplus this past year and flu experts again confronted warning signs of the next flu pandemic, AIDS vaccine experts might have considered taking notes. Experts say these unfortunate episodes provide valuable case studies highlighting the precarious nature of vaccine manufacturing, the difficulty of forecasting demand for biological products, and the challenges of ensuring an adequate supply. Many of the lessons flu experts are learning and the solutions they are proposing could apply to HIV/AIDS should an efficacious vaccine be developed.

Shortages – and then surpluses

Flu season is awaited anxiously every year for good reason. Influenza kills 36,000 Americans annually and hospitalizes 200,000 more. An estimated 500,000 deaths occur worldwide each year. So when US influenza vaccine demand exceeded supply in the 2000-1 and 2003-4 flu seasons, the industry primed itself not to be caught off guard again. The 2004-5 season was supposed to have the largest vaccine supply yet—100 million doses.

Chiron Corporation, one of only two inactivated flu vaccine producers for the US, was expected to supply 48 million of those doses. But the company’s Liverpool, UK vaccine-manufacturing facility had a history of Food and Drug Administration (FDA) inspection deficiencies. On August 25th, 2004, Chiron informed the FDA that it had found Serratia marcescens bacterial contamination in eight lots of vaccine. During September, the company retested its lots and investigated its manufacturing processes. It informed the FDA that retesting results were negative and that the company would fulfill nearly all of its planned supply to the US.

So the October 5th announcement by the UK’s regulatory authority, the MHRA, of a suspension in Chiron’s vaccine licensing came as a surprise to the FDA, which had conducted its oversight by conference call since the first reports of contamination at the facility. After the announcement, the FDA sent a team to inspect the manufacturing facility. It found that some deficiencies noted during the previous inspection in June 2003 had not been remedied, including at least one sterility problem. In mid-October 2004 the FDA determined that it could not assure that Chiron’s finished lots of vaccine met US safety standards, and none of Chiron’s vaccine was allowed to enter the US commercial market. To add to the confusion, this announcement led to contradictory calls—for more lenient regulations to allow other suppliers to rapidly license their vaccines for distribution in an emergency situation and, at the same time, for tougher regulation in order to avoid future manufacturing contamination.

The rejection of Chiron’s entire contribution to flu vaccine supply instantly sliced the number of vaccine doses available to US consumers to nearly half. In response, the US Centers for Disease Control and Prevention (CDC) recommended restricting influenza vaccination to adults and children most at risk for severe complications or death from influenza and others with a high potential of spreading influenza to these vulnerable populations, such as healthcare providers and household contacts of infants under six months of age. In coordination with state and local health authorities, Sanofi Pasteur (previously known as Aventis Pasteur)—the only other US supplier of inactivated influenza vaccine—redirected its remaining shipments to target providers serving high-priority patients. Still, providers were free to dispense shots as they saw fit, and early in the vaccination season there were reports of price-gouging. As during previous shortages, those willing and able to pay these elevated prices had a better chance of receiving their immunizations.

But many Americans heeded the CDC’s advice and if they weren’t in a high-risk category went without a flu shot. As a result of this civil obedience, an early vaccine shortage later turned into a surplus. As of January 2005 more than five million shots were still unsold.

Lessons learned

Chiron’s particular problems were triggered by bacterial contamination during the laborious embryoated chicken egg-based manufacturing process used to grow influenza virus. However, the resulting vaccine shortage stemmed from the fact that such a large proportion of the US influenza vaccine supply was produced at a single Liverpool facility. “I suppose the final point is not to put all your eggs in the same basket with HIV and distribute production in a variety of centers. It was a serious misjudgment for the USA to have 40% or so production in one unit,” says John Oxford of the University of London.

“In any situation where a large component of the supply of something is being produced at a single location there is the potential for natural or man-made events to disrupt that supply,” says John Treanor of the University of Rochester Medical Center. A general lesson that vaccine experts draw from this debacle is the need for a larger, more diverse group of vaccine suppliers.

Unfortunately, the trend in the vaccine business is exactly the opposite. A shrinking pool of manufacturers is becoming responsible for meeting a growing vaccine market. For flu vaccine, for instance, there were about 20 million doses per year distributed in the mid-1980s, compared with an expected 100 million doses for the 2004-5 season. Even with this increasing demand many manufacturers dropped out of the market, leaving only Chiron (Fluvirin) and Sanofi Pasteur (Fluzone) to supply inactivated influenza vaccine. The production of FluMist, a live attenuated flu vaccine manufactured by MedImmune Inc., was also scaled up for 2004-5 but
was only scheduled to produce a relatively small 3 million doses.

“I think that all vaccines are potentially vulnerable to supply disruptions, and I expect that this will also be true of AIDS vaccines, when they are developed,” says Treanor. “Having multiple sources of supply can help to protect against such disasters.”

The problem is finding those sources. The flu vaccine crisis serves to highlight a widespread problem. “On a global scale the experience points out the fragility of vaccine supply in general,” says Stanley Plotkin, emeritus professor at the University of Pennsylvania and an executive advisor to Sanofi Pasteur. From 1966 to 1977, half of all commercial vaccine manufacturers left the market. Now, only five manufacturers—GluacoSmithKline, Merck, Sanofi Pasteur, Wyeth and Chiron—produce all vaccines for the US market that are recommended for routine child and adult immunizations. As of 2003, eight important vaccines for US consumers were each made by a single company—measles/mumps/rubella, tetanus toxoid, tetanus/diphtheria, inactivated poliomyelitis, varicella, pneumococcal conjugate (PCV-7), meningococcal, and pneumococcal polysaccharide (adult). The reason companies are either leaving or reluctant to enter the vaccine market is no secret: vaccines are seen as a risky business. Vice President for New Business and Scientific Affairs of Wyeth, Peter Paradiso, testified on this subject at a Senate Special Committee on Aging Hearing in November 2004. “It has become increasingly difficult to justify remaining in the vaccine business,” he said. Wyeth recently pulled out of both the influenza and DTaP (diphtheria, tetanus and acellular pertussis) markets. Wyeth’s departure from influenza vaccine manufacturing following the 2002-3 season was straightforward. The company’s influenza business had experienced four out of five years of financial losses associated with millions of unsold doses and rising costs related to meeting regulatory requirements.

The problem is that while the need for vaccines may be great, the demand for the product and the ease with which it can be brought to market can fluctuate, leaving companies with product they can’t sell.

Defining demand

The past year’s whipsaw in supply threw production for the following year’s influenza season into disarray. As of January 2005, a critical month for planning the next season’s influenza vaccine supply, it was unclear which manufacturers would supply the US vaccine market and how much vaccine they would be willing to manufacture. There was also the question of uncertain demand. Healthcare providers and vaccine distributors alike held off on placing orders. Some questioned whether low-risk patients would line up again for influenza shots after doing without vaccination during the 2004-5 season.

FluMist manufacturer MedImmune Inc. also held off on committing to a number of doses for next year’s season. The company had initially indicated it was capable of producing 10–40 million doses for the 2005-6 season. On the other hand, manufacturers GluacoSmithKline PLC and ID Biomedical Corp were interested in supplying the influenza vaccine market, but had to wait for FDA approval. And it wasn’t until March 2nd 2005 that British health officials gave Chiron permission to resume vaccine production in Liverpool.

This was not the first time that uncertain demand plagued the influenza vaccine market, as it could well plague the market for an AIDS vaccine (see IAVI Report, 8(1), 2004: “Breaking the Bottleneck”). Even in years when ample influenza vaccine existed, high-risk patients had poor coverage rates. Public campaigns to encourage immunization have contributed to some improvement in vaccination rates. Still, according to results of a National Health Interview Survey, only 43% of US households reported having obtained influenza vaccine, as compared with a target rate of 90%.

For that reason, policy wonks are conjuring up incentives to lure industry back to the vaccine game. For example, in 2003 a committee of the Institute of Medicine, a respected health advisory body associated with the National Academy of Sciences, proposed a system of subsidies and insurance coverage mandates for vaccines.

Increasingly governments, international financial institutions, and other sponsors are proposing to counterbalance the uncertain demand for both influenza and, one day, HIV immunization by strengthening markets and ensuring sales. So-called “pull” mechanisms such as advance purchase commitments would guarantee that a definite quantity of vaccine will be bought at an agreed price, provided it meets pre-specified criteria. Legislation has also been proposed in the US Senate (S.2038) to offer a tax credit to companies constructing or renovating vaccine-production facilities.

Problems encountered last year also underlined the need for improved cross-border vaccine regulatory cooperation—an issue that was raised in the recently published Global HIV/AIDS Vaccine Enterprise strategic plan since any AIDS vaccine will likely need to be manufactured and distributed regionally.

Caution on egg-based technology

Contamination is a constant worry with the egg-based influenza vaccine production process. Eggs are not sterile and because each hen typically can lay only one egg per day, ensuring adequate egg supply is challenging. At three eggs per influenza vaccine dose, producing for the US market requires hundreds of millions of eggs per year. In order to secure adequate supplies of eggs, manufacturers need to forecast yearly vaccine demand six to nine months in advance.
of each flu season. This makes it impossible
to respond to emergencies.

New influenza vaccines must be produced
each year because the influenza virus, like
HIV, quickly changes its genetic stripes. The
influenza surface proteins hemagglutinin
(HA) and neuraminidase (NA)—antigens tar-
geted by traditional influenza vaccines—are
constantly changing, a process known as
antigenic drift. More marked and sudden
genetic reassortment leads to new virus sub-
types and the risk of pandemics and is
known as antigenic shift.

The complexity of the egg-based manu-
facturing process requires more stringent
regulatory processes, including FDA review
of test results on each lot of influenza vac-
cine prior to commercial release. To over-
come some of these challenges, particularly
ensuring sterility and the ability to respond
quickly to changes in demand, the US
Department of Health and Human Services
is supporting research on cell-culture based
influenza vaccines, though at only half the
budgetary level proposed in 2004. An
influenza vaccine composed entirely of HA
proteins and manufactured using a protein
expression system could also shorten the
time between identification of new strains
and vaccine production. A vaccine pro-
duced using a recombinant baculovirus
expression system in insect cell lines (not
requiring eggs or animal serum) has already
reached Phase III trials.

Other researchers, including Andrew
Pekosz of Washington University St. Louis,
David Milich of the Vaccine Research
Institute, San Diego, and Walter Gerhard at
the Wistar Institute, Philadelphia, are exper-
imenting with stimulating an immune
response to conserved influenza antigens
like M2 that are not subject to antigenic drift
and shift, which might also induce protec-
tion across influenza strains. It is also
thought that the FluMist live vaccine may
induce a broader immune response than the
traditional inactivated vaccine.

The hope is that these new approaches
could obviate the need for fresh vaccine for-
mulations each year and thus ease the vul-
nerability to supply shortages. “Influenza
vaccines are uniquely vulnerable to this
type of thing because of the need to pro-
duce a new vaccine every year,” says
Trenor. This vulnerability may apply to
some extent to future AIDS vaccines given
that any candidate that proves effective
enough to justify large-scale production will
undoubtedly be a first-generation product.
Subsequently the production process may
well be optimized, or the vaccine immuno-
gens may benefit from tweaking to follow
any trends in HIV genetic evolution or the
emigration of viral clades and recombinants
into new geographic regions. Similarly,
while early AIDS vaccines are likely to
incorporate immunodominant epitopes, it
might turn out that conserved and more
immunogenic subdominant epitopes are
subsequently identified. “If it were to turn
out that it is necessary to do the same kind
of frequent formulation changes for an AIDS
vaccine as we need to do for flu, then one
might see the same situation arising with an
AIDS vaccine,” says Trenor.

Pandemic fears

Flu and AIDS health experts also share
the worry of dealing with pandemic out-
breaks during which production and distribu-
tion would need to be rapidly scaled for
a global vaccination program. For AIDS, the
pandemic is ongoing. For influenza, the
emergence of a pandemic strain is consid-
ered inevitable and overdue. Avian H5N1
influenza virus first emerged in 1997 but has
re-emerged in recent years and made its
way through large regions of Asia. This viral
strain is able sometimes to infect humans in
contact with birds and cause a high mortal-
ity rate and, most worryingly, it seems to
have been transmitted on rare occasion
from human to human. Only the inefficiency
of this transmission between humans seems
to have prevented it from becoming a full-
blown pandemic virus.

In November 2004 the World Health
Organization convened a two-day meeting of
all major vaccine manufacturers to assess
the status of vaccine preparedness for an
influenza pandemic. The troubling conclu-
sion was that should a pandemic strain
emerge, companies wouldn’t be able to
quickly produce vaccine for the commercial
market. Even at full production levels,
worldwide influenza vaccine manufacturing
capacity totals only an estimated 300 million
doses per year. “A new pandemic would
show up the inadequacy of current facilities
to produce enough vaccine for billions of
people, and poor countries would be the
first to suffer,” says Plotkin. Experts predict
a flu pandemic could cause in excess of
200,000 deaths in the US alone.

Effective vaccines have never been avail-
able to counter such outbreaks in the past.
For pandemic influenza, various solutions
are being explored, including the use of
“mock-up” vaccines to practice production.
Because much of the data required for licen-
sure would be gathered with the mock vac-
cine, manufacturers would be able to more
quickly ramp up production of the actual
vaccine when needed.

Novel technologies and tools are also
being explored for use in potential influenza
pandemic vaccine distribution—including
vaccine formulations that do not require
injections and dose dilution. Also,
researchers are using molecular biology
techniques such as reverse genetics in an
time to more rapidly produce vaccine
candidates that match a particular pandemic
virus strain, and to modify the DNA
sequences of particularly pathogenic avian
influenza viruses, making them more suitable
for vaccine manufacturing. Similar approach-
es are being considered in the search for an
effective AIDS vaccine, particularly as there is
a need to develop a vaccine that can be dis-
tributed as quickly as possible.

Some of the challenges faced by compa-
nies such as Sanofi Pasteur, Chiron, and
Canada’s ID Biomedical, which are commit-
ted to developing and being able to quickly
manufacture a pandemic influenza vaccine,
are similar to those faced by AIDS vaccine
developers. These include funding shortfalls,
potential low return on investment, limited
production capacity, liability fears, and
licensing and regulatory concerns. Both pan-
demic influenza vaccine developers and
AIDS vaccine developers face risk—for
influenza, early production of a pandemic
vaccine prior to the knowledge of whether a
pandemic would occur involves a significant
degree of risk. For HIV, as for pandemic
influenza, risk also centers on the need to
build and validate manufacturing and
process development capacity at least five
years before commercial production, at a
time when efficacy testing is incomplete.

“Lessons for HIV vaccines are that produc-
tion facilities will have to come on line
years before a vaccine has been licensed, and
that those facilities will have to be planned for
a presently unknown demand,” says Plotkin.
“The problem of who will pay the difference
between cost of production and price in
developing countries remains to be solved.”

The influenza vaccine shortages resulting
from contamination at Chiron’s Liverpool
plant and the specter of the next flu pan-
demic have served as a wake-up call,
reminding the public and elected officials of
the importance and fragility of vaccine pro-
duction. With the potentially devastating
influenza pandemic looming and millions of
deaths expected from the existing pandemic
of HIV, experts say the need to increase sup-
port for vaccine research and manufacturing
is more urgent than ever.

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A therapeutic dendritic-cell vaccine for chronic HIV infection

Therapeutic AIDS vaccines attempt to bolster the virus-specific immune response in individuals who are already HIV infected. In a recent report (Nat. Med. 10, 1359, 2004), a group led by Wei Lu and Jean-Marie Andrieu present a preliminary study on the efficacy of a therapeutic AIDS vaccine based on dendritic cells (DCs).

The vaccine comprised autologous activated DCs obtained from the volunteers themselves and incubated in vitro with aldrithiol-2 (AT-2)-inactivated autologous HIV-1 isolates—in essence, each volunteer received a ‘personalized’ vaccine. DCs were cultured ex vivo with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4, AT-2-inactivated virus was added and then cultured with IL-1β, IL-6, and tumor necrosis factor (TNF)-α. The 18 inoculated adult volunteers were HIV-infected for at least a year, with a stable mean plasma viral RNA load of 3 x 10^5 copies/ml and a mean CD4+ T cell count of 550 cells/μl at the time of injection. All patients were immunized three times at 2-week intervals with the AT-2-inactivated HIV-pulsed DCs, and followed for one year thereafter without antiviral therapy.

After vaccination, the plasma viral RNA loads of 8 individuals decreased from around 3 x 10^5 copies/ml to approximately 1 x 10^5 copies/ml during the first 112 days and remained stable for the rest of the year. There was a lesser and transient reduction in plasma viral loads in the other 10 subjects, and in some cases they exceeded preimmunization values one year after vaccination. But in the individuals who did show a sustained reduction in plasma viral loads there was a positive correlation with the numbers of HIV-specific IL-2- or interferon-γ-expressing CD4+ T cells, which normally decline in the course of untreated infection, and with Gag-specific perforin-expressing CD8+ (effector) T cells. Neutralizing antibody apparently did not contribute to reduction in viral loads since titers remained unchanged in 16 of the 18 subjects.

This study in humans is a follow-up to the authors’ previous publication describing a similar vaccine modality (autologous DCs pulsed with AT-2-inactivated autologous virus) in Chinese macaques with chronic SIV infection (Nat. Med. 9, 27, 2003). In the human study the authors could not rule out a nonspecific ‘adjuvant effect’ of the DC preparation since a control group inoculated with activated DCs not pulsed with HIV was not included. However, in the macaque study an appropriate control group treated with pulsed DCs alone (no AT-2-inactivated virus) did not present an adjuvant effect. The authors suggest that unloaded mature DCs cannot present the virus already present in these monkeys, because as mature DCs they have almost lost their antigen-uptake capacity. However, it remains to be seen just how robust these latest results are since the characterization of the individual’s immune responses was minimal, with patients only sampled at 3 time points after vaccination.

An expensive, time-consuming autologous vaccine will never be practical, but this study provides the first demonstration in humans that an HIV-specific cellular immune response can have a positive effect on some parameters of immunity in vivo, and is possibly encouraging news that could be applied to develop more effective, conventional preventive vaccines (e.g., DNA or recombinant viral vector vaccines) that induce similar HIV-specific immune responses in vivo.

The influence of a chemokine gene number on susceptibility to HIV/AIDS

Analyses of sequences of the human genome have identified a large amount of interspersed as well as tandem low-copy repeats or segmental duplications, and the possibility has been raised that variation in the copy number of specific host defense genes may affect the susceptibility to, or the progression or severity of diseases in which the genes play a role.

The copy number of the gene encoding CC chemokine ligand 3-like 1 (CCL3L1) varies among individuals. Importantly, CCL3L1 (also known as MIP-1βP) is the most effective known ligand for the HIV coreceptor CC chemokine receptor 5 (CCR5), and the most active naturally-occurring inhibitor of HIV entry known. To test the hypothesis that segmental duplications of host defense genes causing dosage effects are associated with phenotypic effects in vivo, Sunil Ahuja and his collaborators (Science 307, 1454, 2005) have determined the distribution of chemokine gene-containing segmental duplications in humans and the relationship to HIV infection/susceptibility.

The researchers determined the average number of CCL3L1 gene copies in more than 5,300 HIV-infected and -noninfected individuals of different ancestral origins to see if copy number is associated with either the risk of acquiring HIV or the rate at which HIV disease progresses. They found that increasing CCL3L1 copy number was positively associated with ligand secretion, which is not necessarily the case with all genes. People with different geographical ancestries possessed a significantly different number of CCL3L1 gene copies. This does not mean that any one group is more susceptible to HIV/AIDS than other populations. Rather, using the average CCL3L1 copy number as a reference point for each group, the researchers found that individuals with a CCL3L1 copy number lower than their population-specific median were at a higher risk of acquiring HIV infection.
Study identifies genes that play key role in the immune response against HIV

In humans, human leukocyte antigen (HLA) class 1 molecules are expressed on the surface of cells where they help the immune system recognize virus-infected cells. When new virus particles are produced within an infected cell, class 1 molecules capture fragments of viral proteins and expose them at the cell surface, alerting CD8+ T cells that the cell is infected and should be lysed. Three genes (HLA-A, HLA-B, and HLA-C) encode class 1 molecules. HLA-B genes are extremely diverse, with 563 different alleles identified as opposed to 309 for HLA-A and 167 for HLA-C.

An international research team led by Philip Goulder has identified immune system genes that appear to play a key role in the immune response against HIV (Nature 432, 769, 2004).

The study was designed to test the hypothesis that the diversity of HLA class 1 molecules could reflect functional differences in the CD8+ cytotoxic T lymphocyte responses controlled by those molecules. The authors studied 375 HIV-infected, treatment-naïve individuals in southern Africa to determine if a particular type of class 1 molecule controls the CD8+ T cell response against the virus. To study the contributions of individual HLA class 1 molecules they used a panel of 410 overlapping synthetic peptides, spanning the entire expressed HIV genome, and characterized the T cell responses to these peptides in interferon-γ ELISPOT assays. They found that the association of HLA-B alleles with peptide-specific responses far exceeded that of HLA-A and of HLA-C alleles, so HLA-B alleles contribute significantly more to the total HIV-specific CD8+ T cell response in the studied population.

Next they studied the influence of class 1 molecules on plasma viremia in 706 chronically HIV-infected treatment-naïve persons, and found that viral load varied significantly according to the particular HLA-B allele expressed. To further test whether it is a particular HLA-B allele that principally influences disease outcome, they looked at levels of the CD4+ T cells that are destroyed by HIV in relation to HLA type. The tests all supported the conclusion that the form of the HLA-B molecule inherited by patients makes a significant difference in how well their immune systems cope with the infection.

Finally, they analyzed HLA-B alleles in HIV-infected mothers and their infants. They found that HIV-infected women who have a particular HLA-B allele are more likely to survive, and also less likely to transmit the virus to their infants, suggesting that HIV may be exerting selective pressure on certain protective HLA-B alleles.

Although these data indicate a dominant role for HLA-B alleles in HIV infection the authors are quick to point out that the underlying mechanism remains to be elucidated. They speculate that a possible mechanism is the greater diversity of peptides bound by HLA-B alleles, which can accommodate various positively and negatively charged residues, while HLA-A binds only hydrophobic residues. The study has implications for AIDS vaccine research because it may lead to ways of circumventing the virus’ ability to avoid vaccine-induced immunity by rapid mutation. It also describes how HIV infection may be driving human evolution, since individuals with protective versions of those genes are more likely to survive and pass the genes to children.

Two crystal structures may help guide vaccine and treatment approaches

The envelope glycoprotein of HIV, gp120, attaches to specific receptors and co-receptors on the surface of cells, enabling virus entry. Several years ago scientists uncovered the structure of gp120 bound to the CD4 cell receptor. The structure of fragments of gp41 in its post-fusion state is also known. Now, Stephen Harrison and colleagues (Nature 433, 834, 2005) have determined the unbound monomeric structure of the gp120 molecule, allowing them to build a model predicting that gp120 significantly changes its shape after binding CD4, enabling the molecule to present different antigenic sites in its two states. In its natural state, gp120 is assembled as trimers in the virus spikes together with gp41, also in a trimer.

Researchers have sought the structure of unliganded gp120 for almost two decades and, in a Nature commentary, Peter Kwong describes this work as a “technical tour de force.” To crystallize the gp120 molecule they used gp120 ‘cores’ of the closely related simian immunodeficiency virus (SIV). These cores had the variable loops V1, V2 and V3 deleted, as well as parts of the molecule’s carboxy and amino terminals. Unresolved is the question whether the unbound gp120 structure described here is different from the unbound structure of gp120 in the viral spike. But they were able to crystallize the SIV gp120 cores with their full complement of surface glycosylation, revealing the extent to which these sugar moieties coat the molecule and possibly protect antigenic sites from antibody attachment. They found, for example, that amino acids that compose the chemokine-receptor site on gp120, which forms after CD4 binding, are not contiguous in the unliganded structure, but amino acids involved in mutations that generate resistance to entry-inhibiting compounds are all arranged facing a well-configured pocket.

Vaccine designers will want to know if the bound and unbound partial structures of gp120 now provide information on their antigenic properties that may be helpful to understand the ability of HIV to evade the host’s neutralizing antibody. Arguably, gp120 is more vulnerable in its unbound state before cell attachment, but even in the unbound state alone there may be more than one conformation, when the virus mutates Env to modulate its antigenic structure and escape neutralization. A recent paper by Ronald Montefaro and colleagues (J. Virol. 79, 2097, 2005) emphasizes this ability to evade neutralization by showing for the first time that even point mutations in the intracytoplasmic tail (ICT) of the gp41 can render the virus more resistant to neutralization, seemingly through allosteric effects.

In a second X-ray crystallography paper (Immunity 22, 163, 2005), a group from the Scripps Research Institute led by Dennis Burton and Ian Wilson examined the structure of the broadly neutralizing monoclonal antibody (MAB) 4E10 bound to a peptide fragment identical to the gp41 domain recognized by the antibody. The study identified the amino acids of the epitope recognized by MAB 4E10 as well as its three dimensional structure. Burton and Wilson are members of a consortium of laboratories, the International AIDS Vaccine Initiative’s Neutralizing Antibody Consortium, which is focused on understanding broadly neutralizing antibodies at the molecular level. They hope that the information obtained from the crystal structure will aid scientists in designing immunogens that when used as vaccines could elicit antibodies with properties similar to 4E10.

Research Briefs written by Roberto Fernandez-Larsson
NIH begins second Phase I clinical trial of HIV vaccine

The Vaccine Research Center (VRC), a part of the US National Institutes of Health, started a second Phase I clinical trial in healthy adult volunteers of an AIDS vaccine candidate jointly developed with GenVec Inc. The vaccine consists of recombinant adenoviral vectors that encode the HIV Gag/Pol polyprotein from clade B and HIV Env glycoproteins from clades A, B, and C. GenVec manufactured the adenovirus vector-based vaccine.

The vaccine was developed using GenVec’s proprietary adenovirus vector technology and the 293-ORF6 production cell line. This new Phase I trial will enroll 60 healthy, HIV uninfected volunteers to assess whether administration of the adenovirus vector-based vaccine is safe and well-tolerated in volunteers previously immunized more than one year prior to this study with three injections of a DNA vaccine candidate developed by the VRC. All volunteers will receive the adenoviral vector-based vaccine booster shot after receiving the initial HIV DNA vaccination; 10 additional HIV uninfected volunteers will receive placebo.

The candidate vaccine is also being evaluated in another trial by the VRC in conjunction with the National Institute of Allergies and Infectious Diseases and its HIV Vaccine Trials Network (HVTN). The first VRC Phase I trial, which has completed enrollment, involves a single administration of the adenoviral vector-based vaccine candidate in healthy volunteers.

Results from the study will be used to evaluate safety and to determine if this approach can improve the immune responses against HIV versus responses that are induced by the HIV DNA vaccine candidate alone or the adenovirus vector alone. The secondary objectives of the trial include immunogenicity evaluations and determination of vector antibody titers. Anti-adenovirus neutralizing antibodies could alter the potential of adenoviral vectors to induce immune responses.

—RFL

Human Genome Sciences will start clinical trials with anti-CCR5 monoclonal antibody

Human Genome Sciences, Inc. (HGS) plans to start a Phase I clinical trial to study its investigational new drug, an anti-CCR5 monoclonal antibody (MAb) for the treatment of HIV/AIDS. The CCR5 MAb (CCR5mAb004) is a fully human MAb generated using the Abgenix XenoMouse technology that specifically recognizes and binds the chemokine receptor CCR5, a co-receptor on the surface of lymphocytes that, together with CD4, mediates the binding of HIV and its entry into the cell.

Until recently, the viral reverse transcriptase and viral protease were the only targets of approved antiretroviral agents. The first HIV entry inhibitor, enfuvirtide, was approved in 2003. This class of compounds, known as fusion inhibitors, has received considerable attention, particularly in regard to novel antagonists of CCR5.

Preclinical studies with CCR5mAb004 have demonstrated that it binds specifically and with high affinity to human CCR5, prevents HIV entry, demonstrates no agonist activity or effector functions, and has a long serum half-life. In a 4-week toxicology study of CCR5mAb004, there were no side effects that could clearly be attributed to treatment.

The Phase I clinical trial will evaluate the safety, tolerability and pharmacology of the CCR5 MAb in HIV-infected volunteers in a randomized, placebo-controlled, dose-escalating, multi-center study. The primary objective is to evaluate the safety and tolerability of escalating doses of a single intravenous infusion of the CCR5 MAb. The secondary objectives of the study are to determine the pharmacokinetics of the CCR5 MAb, and to assess its effect on plasma HIV viral load and on CD4+ and CD8+ T-cell counts over time.

—RFL
Phase I trial of ADMVA vaccine begins in New York

The Aaron Diamond AIDS Research Center (ADARC) in New York City recently began enrolling volunteers for a preventive AIDS vaccine trial in partnership with The Rockefeller University and the International AIDS Vaccine Initiative. The Phase I safety and immunogenicity study of the Center’s vaccine, ADMVA, will enroll 48 healthy volunteers in New York City and Rochester, New York.

The candidate vaccine, ADMVA, contains the env/gag-pol and nef-tat fusion genes of HIV and uses a modified vaccinia Ankara (MVA) viral vector. The HIV genes contained in the candidate vaccine are derived from subtype C, prevalent in China, India, and Africa.

“The epidemic in China is burgeoning and the only hope for some people is a vaccine. We are particularly excited about the MVA vector,” said Sarah Schlesinger of ADARC. If results from this trial show the vaccine is safe and effective at inducing an immune response then further clinical trials will be initiated in other regions of the world. For more information visit aidsvaccine@adarc.org

—KJK

NIAID begins enrolling volunteers for Phase IIb study of Merck’s vaccine

Enrollment began in December at sites in the US and Canada for a Phase IIb proof of concept study on Merck & Co’s lead AIDS vaccine candidate, MRKAd5. This efficacy trial will involve 1,500 high-risk volunteers and is being conducted in collaboration with the HIV Vaccine Trials Network (HVTN) and the National Institutes of Allergies and Infectious Diseases. Enrollment will continue in the coming months in Peru, the Dominican Republic, Haiti, Puerto Rico, and Australia.

MRKAd5 is a trivalent vaccine that includes gag, pol, and nef genes from subtype B HIV and utilizes a human adenovirus serotype 5 (Ad5) vector. The trial will test two primary endpoints: the ability of the vaccine candidate to provide protection against HIV infection or control viral load in those newly infected. Volunteers in the trial will receive three vaccinations over a six-month period. Anyone who is incidentally infected with HIV during the course of the trial will be followed to see how the vaccine influences viral load and disease progression.

In earlier studies with the Ad5 construct, immune responses were weaker in individuals who had pre-existing immunity due to previous infection with the naturally circulating adenovirus serotype. This trial will exclude volunteers with an Ad5 neutralizing antibody titer over 1:200, to maximize responses to the HIV antigens.

Previous studies of this vaccine generated strong cellular immune responses in humans. If the immune response in this trial is sufficient to prevent infection with HIV a larger efficacy trial will be required before the vaccine is submitted to the FDA for approval.

—KJK

India’s first AIDS vaccine trial starts

India began enrolling volunteers for the country’s first preventive AIDS vaccine trial in February. The phase I safety study will evaluate the immunogenicity of a vaccine manufactured by the Seattle-based company Targeted Genetics. The National AIDS Research Institute in Pune is conducting the trial, in partnership with the Indian Council of Medical Research (ICMR) and the International AIDS vaccine Initiative.

Thirty volunteers will receive a single immunization with the recombinant adeno-associated vaccine, known as tgAAC09, a recombinant vaccine made from HIV genes enclosed in the capsid of Adeno-Associated Virus (AAV). The AAV vector was developed by Phil Johnson, formerly at the Columbus Children’s Research Institute and currently with the Children’s Hospital of Philadelphia, and is now licensed to Targeted Genetics. The candidate vaccine contains HIV’s gag, protease, and RT sequences and is designed to stimulate both a cellular and humoral immune response to HIV clade C. Preclinical animal studies conducted by Targeted Genetics found that tgAAC09 induced strong cytotoxic T-cell and antibody responses to HIV.

The vaccine candidate is being tested in a joint Phase I clinical trial in Germany, Belgium and now India. The start of the study was hailed as an important advancement in a country with the second largest number of people living with HIV/AIDS in the world. “With this first trial, Indian scientists are making an important contribution that will bring the world a step closer to an AIDS vaccine,” said N.K. Ganguly, Director General of ICMR.

This vaccine trial also emphasizes the critical value of partnerships between governments, private companies, and public organizations in the process of finding an effective AIDS vaccine.

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