

Pursuing Prevention

Are there missing pieces?

Over the past year, the AIDS vaccine field has intensified its focus on discovery and basic research. More scientific questions are being generated than answered, and it's not possible to put these sometimes disparate pieces of knowledge together to "solve" the AIDS vaccine field. Just as with a real puzzle, the number of pieces gives us some idea of how big the "big picture" really is.

How do we handle this pile of pieces, which keeps growing? First of all, by not discarding any piece prematurely. We learn as children that even if it doesn't look like it fits, it might later on, once more has been filled in. In the adult world, this means that the field must continue to balance funding decisions and scientific portfolios so that no single assumption—however cherished—gets a disproportionate investment of time, money or human resources. For example, in the arena of T cell-mediated immunity, where there's ongoing work to define the qualities of an effective HIV-specific response, this means striking a balance between research on epitope specificity and breadth, and research on functionality.

On a larger scale, this means that work on understanding the mechanisms of virologic control must be balanced with research aimed at vaccine-induced prevention of infection. And on an even broader scale, it means making connections between AIDS vaccine research and research on pre-exposure prophylaxis (PrEP) and other proven and emerging strategies. This article looks at some of the areas requiring balance and attention to *all* the pieces.

Piecing together the puzzle of control

Vaccines are among the most powerful public health tools in the world. With a single or a few immunizations, they simply and effectively prevent illness from pathogens such as poliovirus, smallpox, measles, and yellow fever, all of which claimed many lives before effective vaccines were developed. These successes have led to the oft-voiced opinion that an AIDS vaccine that prevents infection would be the single most powerful tool for ending the epidemic. This is true, but it's also true that for some time now, the AIDS vaccine candidates that have reached clinical trials have aimed not at complete protection but at reducing viral load in

people who get vaccinated and go on to become infected. The hope here is to delay time to treatment or HIV-related disease.

This goal has come about because recent candidates have primarily aimed at T-cell immunity, which is often assumed to be incapable of preventing infection on its own. This is suggested by animal model studies and the primary function of cytotoxic T lymphocytes—to kill already infected cells. (This doesn't mean that T cells aren't part of the suite of protective immune responses that an effective vaccine might induce. There are also intriguing hints of T cell-mediated protection, as we discuss later.)

Finding a T-cell vaccine that improves clinical outcomes would be a valuable step toward developing a more traditional vaccine. However, for laypeople and health professionals outside the AIDS vaccine field, it's a major leap from a traditional vaccine to the potential profile of a T-cell vaccine. Impact on viral load setpoint as a surrogate for improved clinical outcome is a very different endpoint than complete or near-complete protection. It's also a major contrast with other interventions, like PrEP or male circumcision or microbicides, where reducing the risk of infection is still the primary goal.

The past few years have seen T-cell vaccines move into efficacy trials. These include the Step test-of-concept vaccine trial, which evaluated Merck's MRK-Ad5 candidate. This study, which looked at the vaccine's impact on both viral load setpoint and HIV acquisition, ended in 2007 when a planned data analysis showed no evidence of benefit, and a potential for increased risk of infection in specific subgroups (for more on Step, see page 32).

The NIH Vaccine Research Center's (VRC) DNA prime/Ad5-vectored boost combination is next to be evaluated in



Clinical Research Continues

Throughout this section, you'll find quotes from AIDS vaccine trial sites in Africa describing some of the work they've undertaken in the past year.

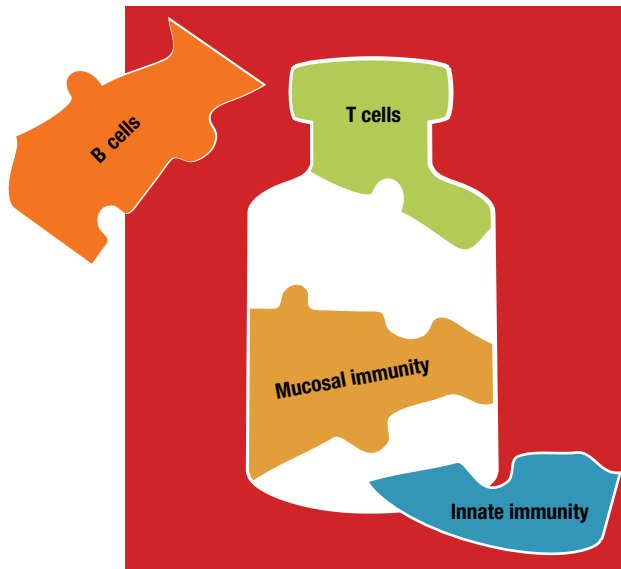
While the field is focused on a range of fundamental basic scientific questions, clinical trials continue to play an important role in vaccine discovery and development. At clinical trial sites, where plans for specific trials may have changed, there's a varied and vibrant range of activities contributing to the overall search for a vaccine.

As demonstrated in our annual table of *Ongoing Vaccine Trials* on pages 42-43, there remains a long list of products in different stages of clinical development.

Notably, results from the Thai prime-boost vaccine trial are expected in September. With over 16,400 participants, this is the largest AIDS vaccine trial ever undertaken. Despite its size, sponsors have pointed out that it is a "test-of-concept" meaning that if there's any sign of efficacy from the strategy—which consists of a canary pox-vectored candidate and the gp120-based AIDSVAX candidate—there will need to be follow-up studies to confirm and learn more.

If there is benefit, there will undoubtedly be questions about whether it is due to the combination of both vaccines or to a single component. (AIDSVAX alone showed no signs of efficacy in two prior Phase III trials.) AVAC will be providing an expanded discussion of the Thai trial as part of its "Anticipating Results" series which will be published prior to the data release.

Working on the Puzzle of Vaccine-Induced Protection



test-of-concept trial called HVTN 505 (see page 38). The strategy being tested in HVTN 505 isn't being considered for development as a licensed product. Instead, its exploratory goals are focused on how vaccine-induced cell-mediated immunity impacts viral load in people who receive the vaccine and later become HIV-infected. (The vaccine itself cannot cause HIV.) Of note, HVTN 505 will be the first AIDS vaccine test-of-concept trial conducted without an acquisition endpoint.

The focus, in these test-of-concept trials, on altering the course of disease is both complemented and informed by discovery research on mechanisms of control. For example, CHAVI collaborators David Goldstein and Jacques Fellay, both of Duke University, have worked with colleagues to identify variations in the major histocompatibility region of the human genome that help determine virologic control. These data came from genome-wide

association studies of samples from the Euro-CHAVI consortium of cohorts. They reinforced the connection between specific alleles, like B57, and control (see box on page 17 for more on genomics research).

The International HIV Controllers Study, headed by Bruce Walker of the new Ragon Institute, has been another source of insight. The study has developed an innovative approach to researching elite controllers—who it defines as individuals who, without ARVs, have maintained less than 50 copies of HIV per cubic milliliter in their blood over at least a year. The study is also looking at individuals who control the virus at low, detectable levels. Researchers in the consortium are looking to learn from these rare individuals and are studying issues like innate immunity, B-cell and T-cell immunity (with a focus on the role of epitope specificity and breadth), host genomics, and patterns of viral evolution. These interconnected topics are often pursued in siloed agendas, and their integration in this project is commendable. So is the engagement of young scientists, several of whom have emerged as new leaders in the field based on their work with Walker and colleagues.

At the National Institutes of Health (NIH), Mark Connors and his lab have focused on other determinants of control including *in vitro* cytolytic capacity, which they're measuring with a single-cell killing assay. At the Keystone Symposium on HIV prevention this year, Connors compared the cytolytic function of elite controllers' T cells with T cells from HIV-positive people who were not controllers, and samples from individuals immunized with the MRK-Ad5 vaccine. The immune responses from the vaccinees had cell-killing abilities similar to those of HIV-positive people

who were not elite controllers. (This doesn't mean that their immune responses were linked to progression, just that they lacked some of the distinctive characteristics Connors and his team have identified in T cells isolated from elite controllers.) No cell-killing assay has yet to be scaled up or standardized for use as part of standard immunogenicity analyses, but as noted in last year's *Report*, it is an important measure that should be considered and adopted more widely when possible.

One of the subtexts for many of the papers and plenaries around these data has been the emergence of what often seem like distinct schools of thought about the underlying mechanisms of T cell-mediated viral control in elite controllers. Some researchers, like Walker and colleagues, are looking at the role of epitope recognition. Others, like Connors, argue that aspects of functionality like cytotoxicity are more relevant and ultimately more predictive. Both lines of thought are fruitful to pursue, and donors and scientists must guard against funding decisions or research plans that stifle either approach.

The pursuit of prevention

The guiding principle behind all of the work described above is that identifying the elements of the immune profile that are associated with virologic control may yield specific targets for vaccine design. This is logical if one assumes that control mechanisms are the same as the mechanisms underlying prevention. But this assumption is uncertain.

We also don't know whether the immune mechanisms that operate in elite controllers are different from those that may be needed to prevent infection.

There is a narrow window when HIV is confined to the genital tract and could, in theory, be contained and even cleared by the right defenses.

So, in addition to keeping a balanced portfolio and a wide-open mind about cell-mediated mechanisms of viral control, it's also vital to maintain, assess, and strategically expand investments in vaccine strategies aimed at prevention.

When prevention does arise in the AIDS vaccine field, it is most frequently linked to potent, neutralizing antibodies—one of the holiest of “Holy Grails” that the field is seeking. The International AIDS Vaccine's (IAVI) Neutralizing Antibody Consortium, some of the consortia of the Bill & Melinda Gates Foundation-funded Collaboration for AIDS Vaccine Discovery (CAVD), and the National Institutes of Health's Center for HIV/AIDS Vaccine Immunology (CHAVI) and the VRC have all continued efforts to identify and isolate broadly neutralizing antibodies.

They've also worked on designing antigens that mimic transiently exposed targets on the virus. There's growing interest, too, in harnessing innate immune responses to help stop infection at the earliest points of entry into the body. However, the timeframes for developing candidates for clinical trials based on this work are long and the challenges are many.

One of the more interesting questions that we've heard this past year, which frequently emerges in discussions about all that's being learned about the multifaceted mechanisms of virologic control, is this:

Is the vaccine research agenda, which has focused in recent years on the immunobiology of viral control through T-cell vaccines and other work, in the best shape possible to fully explore the potential for preventing infection?

This question is easier to raise than to answer. The science of protection from HIV infection hasn't had as many advances as that of viral control; the latter being easier to study given appropriate resources for screening and identifying the right cohorts.

In addition, the borderline between the studies of prevention and control is fuzzy at best. Information about transmitted viruses and about the immune defenses that get mounted in the very early stages of established infection can provide critical clues for design of both preventive and disease-slowng vaccines. Likewise, studies of people with HIV have yielded rare but potent neutralizing antibodies. There's also more recent data from Scheid et al.¹ suggesting that several type- or strain-specific antibodies with limited neutralizing abilities individually can be combined to achieve effective *in vitro* viral control.

However, there are some novel ideas worth considering, such as the possibility of quelling a localized infection in the genital tract before it spreads and establishes systemic infection. To explain how this might work, Robin Shattock of St George's Hospital at the University of London uses the comparison of installing a sprinkler system to control a small fire, versus pulling up fire trucks to extinguish a roaring blaze. In this case, the small fire is the localized infection that HIV establishes

in the cells of the genital tract in the very first hours of infection. HIV spreads very rapidly from the site of entry to the rest of the body. However, there is a narrow window when it is confined to the genital tract, where it could, in theory, be contained and even cleared by the right defenses present in the right quantities at the right time.

The fire-truck approach is how Shattock describes work on vaccines aimed at blunting early viral damage and viral load. A vaccine that induces defenses aimed at control is targeting HIV as it is establishing infection. The aim of this type of vaccine isn't to clear the virus but to control it. This encompasses possible effects including blunting peak viremia, lowering viral load setpoint, or helping to achieve durable control without treatment, all of which might lay the foundation for better outcomes by preserving immune responses.

But there's also been a surge of interest in T cell-mediated clearance of local infection, since data presented by Louis Picker et al.², which report complete protection observed in four out of 18 rhesus macaques immunized with a replication competent RhCMV-vectored vaccine. Picker and colleagues identify cell-mediated immunity—specifically effector memory cells in the mucosa—as the primary mechanism of protection. They suggest that the animals were infected with SIV and managed to clear the local infection before it spread. This argument is shored up by the fact that the protected animals had SIV-specific immune responses to antigens that were

¹ Scheid JF, et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. *Nature*. 2009 Apr 2;458(7238):636-40. Epub 2009 Mar 15.

² Hansen SG, et al. Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nat Med*. 2009 Mar;15(3):293-9. Epub 2009 Feb 15.

not contained in the vaccine—an indication that they had “seen” the virus, even though they were not infected. This is a single, small animal study that has to be confirmed and further clarified, but it’s intriguing nonetheless, both for its

suggestion of protection and because it underscores the importance of looking at mucosal immune responses.

If a vaccine were to help control infection at the local site it would have to win what



Using Genomics to Generate New Hypotheses

Across the field, entities like CHAVI, Bruce Walker’s group at the Ragon Institute, the French research agency ANRS, and others are using genome-wide association studies to look for regions of the human genome associated with viral control or disease progression. Participants at a January NIH-convened meeting on genomics and HIV underscored that these genes, while important, might be different from those associated with protection. One innovative approach to identifying protective alleles: a proposed CHAVI study of HIV-positive and HIV-negative hemophiliacs known to have been exposed before stringent blood bank controls were introduced.

At the NIH genomics consultation, there was great interest in whether ongoing HIV prevention research trials, such as PrEP studies—which will enroll over 17,000 individuals by mid-2009 (see article, page 46)—could aid this effort by contributing samples and helping to identify individuals in the earliest days of infection. “We are especially concerned that observational or prevention studies already underway be examined for their utility in informing genetic associations with transmission mechanisms,” noted the meeting working group, on the host immune response and susceptibility to HIV chaired by Myron Cohen of the University of North Carolina.

What information can we glean from current studies, and what are the barriers? This is an area where immediate action could clarify opportunities swiftly and relatively easily. In some ongoing studies, participants give permission for samples to be used for genetic analyses; in others, no explicit permission is given. Different studies have different schedules for HIV testing and/or viral load monitoring in individuals who become infected. The field needs to review procedures in different trials and look across biomedical prevention research to determine what’s possible to standardize in sample collection.

There’s also a need to explore novel designs for studies in humans, where the timing of sampling is more frequent. The US Military HIV Research Program (MHRP) is working on a protocol in this regard that involves biweekly blood draws for rapid turnaround HIV nucleic acid testing to identify acute infections and to compare the host genetics of those who are at risk of infection and become infected versus those at risk who do not become infected.



Big Questions for 2009 and Beyond

AIDS vaccine research has many enduring questions: Does clade matter? What constitutes an effective immune response? How can we best use the non-human primate (NHP) animal model? In these pages, we look at some of the recent research on these topics, but our *Report* is far from exhaustive. Below are some important questions that we hope will be addressed in the coming year and beyond.

1. What are the roles of various characteristics like epitope specificity and functionality in CD8 T cell-mediated viral control?
2. How does HIV genetic diversity matter for vaccine research? What systematic attempts can be made to address the relevance of clade—and at what stage in the vaccine discovery process should these take place?
3. What future directions best guide improvements in animal modeling efforts? Are current NHP vaccines and challenge viruses sufficiently predictive given their limitations?
4. What are the next steps in learning about immune activation and its impact on susceptibility to HIV infection? What clues can be gleaned from studies of nonpathogenic non-human primate models, like African Green Monkeys? How can this be applied to vaccine design?
5. If combinations of many antibodies are necessary for protection, is there a definable set?
6. What needs to happen to optimize progress in the study of adjuvants, especially for DNA products or toll-like receptors (to harness innate immunity)?
7. How can studies of candidate vaccines best elucidate the contribution of more than one type of immune response to protection?
8. Will vaccine studies evaluating candidates for their impact on virologic control take sufficient account of the effects of viral persistence and latency?

University of Minnesota scientist Ashley Haase calls the “race between the virus and the host,” which starts the instant HIV penetrates the mucosa and begins to infect or be engulfed by cells of the immune system. Within hours, HIV spreads from the local mucosal sites of exposure to the

lymph nodes. There are cells present in the local mucosa that try to block HIV, but they’re there in insufficient quantities—outnumbered by the viral particles. (This mechanism has limited relevance to exposure via injection drug use in which there’s no mediating mucosal barrier.)

Haase has put forth an “enough, soon-enough hypothesis.” This proposes that a vaccine might reduce risk of infection by inducing sufficient responses at the mucosal sites of sexual exposure to stop HIV from expanding to a systemic infection.

Haase doesn't limit his thinking to what vaccines can do alone. Microbicides could also play a role by blocking the receptors on the local mucosal cells that HIV targets early in infection, or perhaps, by delivering ARVs that block or slow viral replication, delaying the spread from local sites to the bloodstream. In this scenario, PrEP or a microbicide (both ARV- and non-ARV formulations) might buy time for vaccine-induced immune responses to expand and swing into action.

It's a concept that some are calling biological synergism: pursue prevention by combining different biomedical interventions with complementary mechanisms. One theory, which is yet to be tested, proposes that ARV-based prevention plus an effective vaccine strategy could provide even better protection than PrEP alone or could be a way to enhance and back up PrEP effectiveness for people who may not take PrEP dosing as scheduled or prescribed (see page 46 for more information about PrEP and ARV-based microbicides).

There's also the possibility of combination approaches that aim to prevent infection and also to provide improved control of the virus if infection does take place. There are animal data to suggest that PrEP might work this way, i.e., blunting viremia in people who get infected while taking the drug. And improved virologic

control is one of the primary endpoints being measured in T-cell vaccine studies. (As discussed on page 34, there's also a faint glimmer that a small subset of vaccine recipients in the Step vaccine trial might have had some level of vaccine-induced virologic control.)

Innate immunity might also play a critical role in protection, both as a primary defense and mechanism through its modulation of adaptive immunity. This assertion has more specificity behind it thanks to work from Bali Pulendren and Rafi Ahmed of Emory University, and collaborators, who have used systems biology approaches to begin to decode the complex, integrated immune responses induced by the highly effective, licensed yellow fever vaccine.

When it comes to prioritizing prevention as a goal for AIDS vaccines there are some broad areas where additional work could be done. The Global HIV Vaccine Enterprise, which is currently revising its

“Science dictates that we change as the field changes. When the Step results were released, KAVI had to make some adjustments. With no HIV vaccine candidates immediately available for testing, we have focused increasingly on participating in a series of basic research and epidemiological protocols. The highlight of these is a multi-country study looking for the presence of neutralizing antibodies in HIV-positive individuals not on ART and not progressing to AIDS, which may inform vaccine design. We hope KAVI's contributions to these studies may help answer some of the fundamental questions in HIV vaccine development.”

Prof. Walter Jaoko, Principal Investigator, Kenya AIDS Vaccine Initiative, Kenya

Scientific Strategic Plan (see page 24), is well-positioned to catalyze and monitor some of this work:

- **Validating and expanding on recent scientific developments**

- Repeat, validate, and explore mechanisms underlying findings from non-human primate models, including the evidence found by Picker et al. of protection with an RhCMV-vectored vaccine and the work by David Watkins's group using heterologous challenge stocks with limited viral diversity.³ (At the same time, there is a need to explore regulatory feasibility of moving replication-competent vectors like RhCMV into humans.)
- Continue to follow directions suggested by immunogenicity data from human trials of licensed vaccines, such as those generated by Pulendren and Ahmed.
- Follow up on the various hypotheses regarding mechanisms of control by

elite controllers, including those advanced by Walker and David Heckerman regarding epitope recognition, and Mark Connors's work on cell killing.

- Ensure that, where possible, mucosal samples are collected and analyzed to provide clues to immune responses in humans and non-human primates. One avenue for this could be the new HVTN/CHAVI initiative that aims to foster interaction between non-human primate researchers and clinical researchers.
- **Field-wide goal setting aimed at taking on prevention as a goal for the AIDS vaccine field**
 - What are the options for gathering samples that might help individuals who are protected from, or manage to clear, a localized infection? Can timing of sample collection (i.e., after sex or unplanned exposure) be used to reach these goals?
 - What are the resource needs in terms of funding and organizations to pursue these questions? Are there gaps based on current contributions from IAVI, NIH, the Gates Foundation, amfAR, and other donors?
 - How can the current CHAVI and CAVD groups contribute, and how should the next iterations of these ventures—if they come to pass—be organized in light of these questions? (For more on field-wide organization, see the article on the Global HIV Vaccine Enterprise, page 24.)

“With Phambili closed and PAVE 100 not started, we had to turn to other prevention-related issues, like involvement of adolescents and minors in prevention research, understanding the role of circumcision in HIV prevention, examining the impact of Phambili closure, examining our participatory practices and level of risk-reduction counseling services among other things. At our Emavundleni site, we've taken on ‘poly prevention’ and have diversified our prevention research efforts in order to keep interest and activity.”

Linda-Gail Bekker, Principal Investigator, Desmond Tutu HIV Foundation, South Africa

³ Watkins DI. Vaccine-Induced Cellular Responses Control Acute SIV Replication After Heterologous Challenge. *Keystone Symposia Conference: Prevention of HIV/AIDS (X3)*. Keystone, Colorado, 2009 March 22-27. Abstract #005.

All of the above are interconnected like, yes, puzzle pieces. For example, understanding the mechanism of vaccine-induced protection could strengthen the rationale for testing specific vaccines with PrEP.

The good news is that much work is underway, and the diversity of players is striking and encouraging. Haase, unlike Walker and Picker, isn't linked into any of the "big science" consortia like CAVD and CHAVI, but he does receive funding from NIH and IAVI. Louis Picker is part of IAVI's Live Attenuated Consortium, and IAVI is funding some of the follow-up studies, as is CAVD. CHAVI is following a cohort of exposed, seronegative individuals, and its work on acute infection and isolating transmitted viruses is also focused on very early events of transmission.

The CAVD portfolio includes several relevant projects including the work of Julie McElrath of the University of Washington and colleagues on adjuvants that might manipulate the innate immune

Understanding the mechanism of vaccine-induced protection could strengthen the rationale for testing specific vaccines with PrEP.

system. amfAR has supported work out of St. George's University in London using *ex vivo* models to examine how candidate microbicides might enhance colorectal immune responses and/or block viral activity to HIV at six and 24 hours after exposure.

This is a fertile mix of big science and individual efforts, of product-oriented work and of slow and steady basic science. (It's also a reminder that seasoned veterans like Haase, Connors and Walker have as much to contribute as young investigators, and that both cadres are essential.) This is why we'd like to see a quick, strategic, scientific analysis of all the efforts underway, with a goal of identifying gaps and opportunities for synergy both within the AIDS vaccine field and across the vaccine, PrEP, and microbicide fields. ✦