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# IAVI Report

## Contents

10 Microbicides Come of Age?

12 An Interview with Susan Allen

Serodiscordant Couples: Africa's Largest HIV At-Risk Group

16 Editorial

Proof of Concept
New-look *IAVI Report*

17 Research Briefs

19 Vaccine Briefs

# Microbicides in the spotlight

*Recent meetings highlight this young field and its many points of convergence with AIDS vaccines*

**by Emily Bass**

The recent International AIDS Conference in Bangkok will be remembered as the meeting in which microbicides—topical compounds designed to prevent sexual transmission of HIV—attained the spotlight which has frequently eluded this small but growing field over the first two decades of its evolution.

Calls to hasten microbicide research started at the opening ceremony of the conference when United Nations Secretary General Kofi Annan urged the audience of thousands to do more to protect women and girls and ensure "that they have full access to the practical options that can protect them from HIV—including microbicides, as they become available."

Throughout the week, a diverse array of speakers—many from outside the field's small community of longtime advocates and researchers—also singled out microbicides as a crucial strategy for mitigating what Annan called the "terrifying" impact of the epidemic on women and girls (see box, page 6). By the time the vaccine and microbicide "theme" day arrived on the last full day of the conference, Zeda Rosenberg, head of the International Partnership for Microbicides, set aside her prepared plenary text to remark, "when I wrote this presentation weeks ago, I could not have predicted that this would be a watershed conference for microbicides."

The surge of interest in microbicides comes at a time when the field is coming into its own. In recent years the field has been energized by an influx of funding from a variety of sources including the Bill & Melinda Gates Foundation, the Rockefeller Foundation, and European governments. There has also been an awakening of interest among basic scientists who are focusing on determinants and early events of sexual transmission of HIV with an eye to developing effective microbicides.

As with AIDS vaccines, microbicide research faces challenges related to coordination among research entities, long-term planning for success or failure of first and second generation candidates, and the need for clinical trials capacity in resource-poor settings. The

*continued on page 6*

Review

# Early dendritic cell-driven events governing the mucosal transmission of HIV: targets for vaccines and microbicides

**by Melissa Pope PhD**

HIV breaches mucosal barriers as either cell-free or cell-associated virus, subsequently interacting with local leukocytes within the tissues to establish infection. The exact role of cell-free vs cell-associated virus (and which cells within the inoculum are infected) in mucosal transmission remains unresolved. Most research has focused on transmission of cell-free virus, but more innovative investigations are needed to verify and characterize the nature of incoming infectious inoculum. However borne, in penetrating the epithelial surface HIV might either infect epithelial cells, pass between these cells or directly through breaks in the tissue, or transcytose epithelial cells (reviewed in ref 1). Subsequently, multiple white blood cell types within the tissues can be targeted by HIV, initiating the sequences of events facilitating the onset and dissemination of infection. The earliest events of virus-cell interplay, with an emphasis on the particular role of dendritic cells (DCs), will be discussed here, highlighting what needs to be considered to improve vaccine and microbicide strategies to prevent mucosal transmission and dissemination of HIV.

### *Initial leukocyte targets within the epithelial tissues*

DCs, macrophages, and $CD4^+$ T cells can all serve as targets for incoming HIV. Increasing evidence supports the notion that DCs positioned within and just underneath the epithelia are one of the first leukocytes to interact with the virus and that they are critical to the onset of infection (reviewed in refs 1-4) (Figure 1). Like macrophages and $CD4^+$ T cells, immature DCs can be productively infected with CCR5-using HIV (R5 HIV). DCs have the additional attribute that they can also efficiently capture virions (independent of infection) and this, as well as virus newly produced by infected immature DCs, can be rapidly transmitted to $CD4^+$ T cells[5,6]. Macaque studies document that virus-positive Langerhans cells (LCs, the DCs in the outer stratified squamous epithelia of the vagina, anus, and oral mucosa) can be detected within the first 1-2d after vaginal exposure to SIV and virus-positive T cells appear 2-3d after mucosal (vaginal or oral) challenge (reviewed in refs 2, 3). This suggests that DCs entrap virus during the first moments after exposure, possibly replicating R5 HIV themselves (at least at low levels), before passing it to the more permissive $CD4^+$ T cells (and macrophages) that then amplify infection both locally and in distal tissues (Figure 1).

DCs are in an immature state in healthy epithelial tissues, but an influx of DCs and DC maturation occurs in inflamed tissues. Although mature DCs are more resilient to productive infection by HIV, mature DCs readily capture HIV and transfer it to $CD4^+$ T cells driving robust virus replication (reviewed in refs 2, 3). So inflammation caused by other sexually transmitted infections potentially results in mixtures of immature and mature DCs as well as the influx of $CD4^+$ T cells, thereby providing additional cellular targets to increase the chances of HIV transmission. Furthermore, DC activation can occur during migration from the mucosal tissues to the draining lymph nodes. If the migrating DCs are carrying HIV, the activation process could influence the subsequent fate of the virus: infectious virus might be retained for subsequent transmission, *de novo* synthesis of infectious virus might be shut down, or the captured virions might be degraded. Understanding how HIV interacts with different DC subsets (the activation state of which might have been modulated following exposure to other pathogens or factors) is therefore vital to identifying what needs to be tackled to restrict mucosal transmission.

An additional point to keep in mind is that R5 HIV isolates predominate in the earliest stages of infection, despite the likelihood of exposure to both R5 and X4 (CXCR4-tropic) viruses. Although the exact mechanisms controlling this are not understood, the presence of numerous cellular targets capable of replicating R5 viruses might contribute at least in part to this phenomenon (DCs and macrophages are poorly infected by X4 viruses). However, the fact that DCs can capture both X4 and R5 HIV and transmit them to T cells to promote virus growth in the DC-T cell milieu (reviewed in refs 2, 3) suggests that X4 viruses should be able to establish infections *in vivo.* Indeed a recent report showed that upon co-exposure of macaques to a mixture of X4 and R5 SHIVs the animals became infected with both isolates, but that the R5 SHIV quickly dominated and the X4 SHIV receded coincident with the appearance of virus-specific $CD8^+$ T cells[7]. X4 SHIV re-emerged upon depletion of the $CD8^+$ T cells. This suggests that at least one of the governing features includes the differential immune control of X4 and R5 viruses, enabling the outgrowth of R5 virus. The mechanism underlying this differential immune control needs to be elucidated to advance vaccine design—perhaps it is dictated by how the DC "presents" an X4 compared to an R5 virus?

### *Virus-cell interactions in the early moments*

Virus interactions with most leukocyte receptors are largely dependent on virus envelope. While DCs (especially immature DCs) have terrific capacities to non-specifically ingest particles, they also appear to capture virus predominantly by envelope-dependent, receptor-mediated mechanisms[8]. Productive infection of immature DCs with R5 HIV requires CCR5, probably involving the classical CD4/CCR5-dependent binding and virus-cell fusion events (reviewed in refs 2, 3). Down-regulation of CCR5 expression upon DC maturation at least partially explains the more limited infectibility of mature DCs.

More recent evidence underscores how immature and mature DCs can also capture viruses via mannose-dependent C-type lectin receptors (CLRs) such as mannose receptor (CD206), DC-SIGN (CD209), and Langerin (CD207) (reviewed in refs 2, 3, 9-11). CLR-dependent capture of virus is very efficient and may augment CCR5-dependent infection of DCs in *cis*[12]. However, CLR-entrapped virus is mostly internalized by the cells and subsequently transmitted to $CD4^+$ T cells (in *trans*) in the absence of DC infection[6,13]. CLRs are differentially expressed on distinct DC subsets and as a result unique virus-CLR interactions occur with each subset. For instance, LCs lack CD209 but express CD207, while submucosal DCs lack CD207 yet express CD206 and CD209. So as well as being susceptible to infection with R5 HIV, immature DCs express a variety of CLRs that enable proficient entrapment of virus that can then be disseminated.

These two dominant modes of DC-virus interplay (CCR5-dependent and CLR-dependent) are manifest as two phases of transmission of virus from DCs to T cells (Figure 1). Using model monocyte-derived DCs (moDCs) to closely follow the kinetics of retention of infectious virus, we recently demonstrated the transfer of entrapped virus to T cells independent of DC infection (both immature and mature DCs) as well as the transfer of newly-synthesized virions by productively infected immature DCs[6]. Comparable biology has been described using a cervical tissue explant model, where the tissue is exposed to HIV *in vitro* (in the presence or absence of specific blockers)[14]. Actual infection of cells within the tissue can be monitored as well as the ability of the cells that migrate from the tissue—mimicking the migration to the lymphoid tissues—to transmit infection to permissive cells. Cells within the mucosal tissue explants are preferentially infected by R5 HIVs and blocking CD4/CCR5-dependent interactions between the virus and the mucosal cells prevents subsequent infection. In contrast, the ability of the migrated cells to transmit infection is not affected by CD4/CCR5 blockers, but is impaired when CLR-virus interactions within the tissues are inhibited (e.g., with mannan). Of note, the migrated CD3-HLA-$DR^+$ fraction comprising numerous DCs is ultimately responsible for virus transmission.

Despite the considerable involvement of CD4, CCRs and CLRs in the various virus-DC interactions, virus capture by DCs is rarely blocked 100% by blocking strategies targeting these molecules[6,14,15]. This may simply reflect only partial efficacy for these *in vitro* analyses and that we need to identify more effective CD4, CCR, and CLR blocking agents. However, determinants other than CD4, CCRs, and CLRs on DCs also likely contribute to virus-DC interplay and need to be considered when preventing DC-driven HIV spread.

Once the virus is trapped by DCs (by whatever mechanism) it can be very rapidly transmitted to neighboring T cells, exacerbating virus dissemination. Earlier work highlighted the ability of DC-T cell

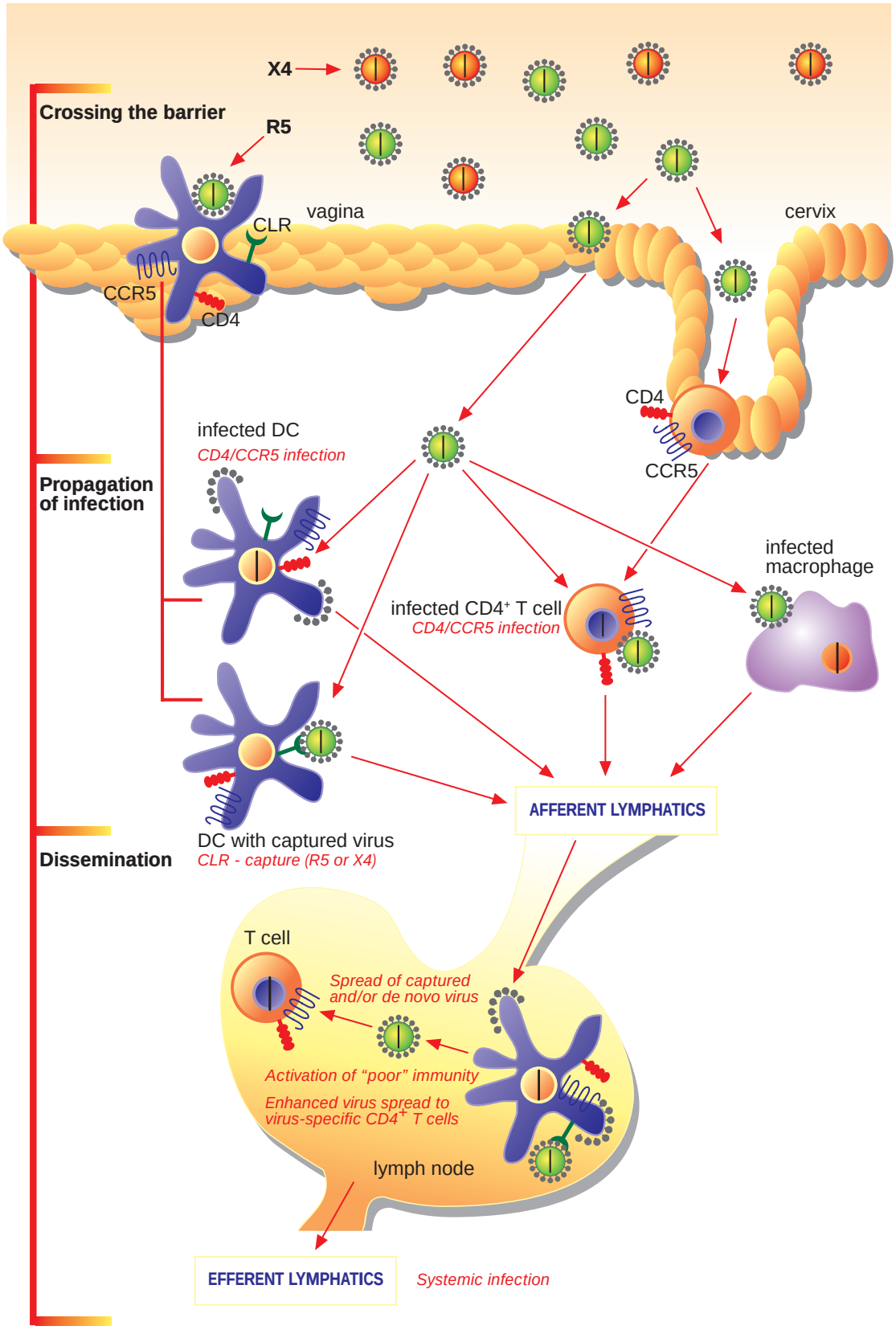


**Figure 1: Early events during sexual transmission of HIV**. *R5 and X4 viruses can cross the epithelia as (i) cell-free virus passing through the barrier and/or upon interaction with cells in the epithelium (DCs, T cells, epithelial cells) or (ii) cell-associated virus (not shown). The exact mechanisms determining why R5 infection dominates is not completely understood. DCs, T cells, and macrophages can be productively infected via a CD4/CCR5-dependent mechanism and DCs capture viruses (potentially R5 and X4) via CLRs. Cell-to-cell spread of virus likely occurs within the local epithelial tissues (not shown). Virus-carrying and -infected cells move via the afferent lymphatics to the draining lymph nodes, resulting in virus dissemination to and amplification in resident CD4 T cells. This is exacerbated by the migrated DCs being able to transmit virus extremely efficiently to T cells (captured viruses and those newly produced by the DCs) while activitating poor anti-viral immune responses. Amplified viruses (cell-free or as infected CD4 T cells) then move via the efferent lymphatics facilitating systemic infection.*

**HIV manages to subvert the antigen-presenting cell system to favor infection instead of robust protective anti-viral immunity**

conjugates to amplify virus infection[16] and more recent studies revealed that virus transmission from DCs occurs preferentially to proliferating T cell subsets[17,18]. Strikingly, virus transfer from DCs to T cells has now been visualized[5,6]. Virus moves quickly to the synapse between the DC and T cell, where CD4 and CCR5 molecules congregate[5]. Once virus has moved from the DCs to the T cells, there is T cell-T cell spread of virus[6] through CD4/CCR-dependent mechanisms[19]. While some virus moving from the donor cell might be newly produced budding virions, whole internalized virus particles are also released at the contact points to fuse with the recipient T cell membrane (unpublished observations).

### *Virus exploitation of the immune system drives early spread*

The natural function of DCs in the immune system is to capture pathogens, present them to the immune system and stimulate potent pathogen-specific immunity. But HIV manages to subvert the antigen-presenting cell (APC) system to favor infection instead of robust protective anti-viral immunity. Both immature and mature moDCs that have captured virus are able to stimulate virus-specific T-cell responses *in vitro* [20-24]. Notably, immature DCs preferentially stimulate $CD4^+$ T cells while mature DCs induce both $CD4^+$ and $CD8^+$ responses[24]. Therefore, when an immature DC entraps incoming HIV, virus-specific $CD4^+$ T cells may get activated, but not $CD8^+$ T cells—a response insufficient to eradicate infection. Additionally, virus-specific $CD4^+$ T cells are more susceptible to infection[25] and so the activation of virus-specific $CD4^+$ T cells might further augment virus dissemination (Figure 1).

Unlike mature DCs, immature DCs typically induce poorer Th1 effector responses, and in fact stimulate regulatory T cell (Treg) responses[26] that may dampen any virus-specific innate or adaptive responses elicited during primary infection. Recent work suggests that Tregs control the immune responses to HIV infection[27] and that natural Tregs are especially susceptible to HIV infection[28]. Additionally, a recent report indicates that HIV-infected immature DCs favor the induction of IL-10 responses that would dampen Th1 immunity[29]. Hence, by targeting immature DCs within the epithelial tissues, HIV avoids the activation of strong effector responses and favors the activation of Tregs, further limiting effective clearance of infection and, if anything, creating an even more permissive milieu for virus replication.

Adding to this is increasing evidence that determinants within the virus can modulate APC functions to drive virus infection while avoiding potent immune activation. Unlike other pathogens, HIV does not stimulate DC maturation and as such limits the likelihood that a virus-bearing DC will elicit strong effector immunity unless an exogenous DC stimulus is provided. In fact, HIV seems to hijack selective attributes of the APC machinery to favor its own replication. HIV Nef triggers DCs and macrophages to secrete chemokines and cytokines to attract additional T cells to the initial focus of infection (reviewed in ref 3), thereby providing more targets for virus amplification. Moreover, Nef-signaled macrophages activate B cells that in turn signal resting T cells to become permissive for HIV infection[30]. Nef-expressing immature DCs also signal resting T cells and drive virus growth[31]. It has been suggested that Nef modulates CD209 expression to promote DC-T cell contact needed to drive infection, although this is not seen in all Nef-bearing DCs (reviewed in ref 3). Despite this, Nef-bearing DCs do not up-regulate costimulatory molecules (an event typical for mature DCs and essential for effective immune stimulation) and therefore remain poor stimulators of anti-viral effector responses. Similar modulation of DC biology in the absence of classical phenotypic activation is also induced by Tat[32]. As a result, HIV factors selectively exploit specific aspects of APC biology to encourage APC-T cell communication and drive virus spread while sidestepping the activation of effector immune responses.

### *Considerations for preventing HIV transmission*

As we learn more about the early events of virus crossing the mucosal barriers, it is clear that we must bear in mind (i) the variety of cells with which HIV interacts, (ii) the multitude of receptors that are utilized by HIV to enable infection and/or entrapment, and (iii) the complexities of the subsequent efficient spread of virus between cells. As reviewed recently[1,33], the immune system is faced with an enormous challenge within a relatively short window of time to control the initial stages of virus amplification. These challenges exist for the development of both vaccines and microbicides against HIV.

Whether it is a vaccine-elicited immune response or a topically applied microbicide, a broad acting strategy is needed to impede the wide array of different HIV envelopes from interacting with all potential cellular targets

(and the various molecules on their surfaces). Anti-envelope approaches should limit most envelope-mediated interactions and act fairly broadly to this end. Passive transfer of neutralizing antibodies (NAbs) protects against intravenous SHIV challenge[34,35], indicating the importance of NAbs in controlling infection. But NAb responses will most probably need to be elicited by vaccines at the mucosal surfaces to have significant impact in preventing transmission[36]. NAbs probably have greater impact by preventing the infection of new targets, while cellular responses will be required to eradicate already infected cells (as in therapeutic strategies for infected individuals). It is vital that vaccines are presented appropriately to the immune system to ensure that potent effector T (and B) cell responses, not regulatory responses, are induced, such as can be achieved through targeting mature DCs[37]. This might also require coordination of boosting innate responses by DCs (e.g., IFN-α, defensins) to assist directly in virus control and also enhance the activation of adaptive responses[37,38]. Thus, vaccines face the challenge of having to induce antigen-specific effector responses with wide specificities in order to clear infected cells and prevent new infections, as well as dealing with the ever-mutating virus.

While still a daunting task, microbicide strategies may be designed to target more generalized features of the virus or even host molecules and thereby be less restricted by the continuously evolving virus. For instance, anti-envelope NAbs protected at least 70% of monkeys against vaginal infection with SHIV[34,39]. These data provide proof of principle that mucosal transmission can be impeded by blocking envelope-host interactions (as well as emphasizing the importance of inducing mucosal Ab responses through vaccination). In agreement with the need for broad-acting modalities, the negatively-charged sulfated polysaccharides like Carraguard (a carrageenan-based formulation) represent a promising approach to potentially interfere with all virus-cell interactions as well as cell-to-cell spread through their (charge-based) non-specific actions. In fact, Carraguard significantly impaired virus capture by immature and mature moDCs (unpublished observations) and protected approximately 70% of the monkeys vaginally challenged with infectious SIV (David Phillips and Louis Martin, personal communication). Not surprisingly, just blocking CCR5 (with a single CCR5 inhibitor) had a less dramatic effect, preventing vaginal SHIV infection in only 2 of 11 macaques[40] (although the viral replication in all animals was reduced compared to the control group). Therefore, broad-acting and/or combinatorial approaches will probably exert the most effective preventive microbicide strategies.

### *In summary*

The primary events of virus-cell interactions following the immediate penetration of the epithelial barrier are multifaceted, involving multiple cell types that express a variety of molecules to bind virus (CD4, CCRs, CLRs, others). Moreover, cell-to-cell spread of HIV is especially efficient and the tight junctions between the cells may afford "protection" for the virus being transmitted, making it difficult to block critical interactions. Adding to this, the immune system is exploited by HIV to foster the stimulation of sub-optimal immunity, further exacerbating the intricacies of the onset of infection. Defining the complexities of these events will help develop vaccine and microbicide modalities with sufficient strength and breadth that are needed to limit HIV transmission and dissemination.

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## HIV and Women: Statistics from a Global Crisis

- Worldwide, women comprise about half of all people living with HIV[1]
- In sub-Saharan Africa, women comprise 57% of all people living with HIV; that figure rises to 75% among young people (aged 15-24)[2]
- In Zambia and Kenya, young married women (aged 15-19) are at significantly higher risk of HIV infection than unmarried young women in the same age group[3]
- Twenty five percent of South African women are infected with HIV by the time they are 22 years old[4]
- Women are being infected with HIV at faster rates than men in many areas of Asia, including the Mekong Region, Cambodia, Laos, Thailand and Vietnam[5]
- From 1985 to 2002, the proportion of adult/adolescent AIDS cases in the United States reported in women increased from 7% to 26%[6]
- In Trinidad and Tobago, the number of women between 15 and 19 years old with HIV is five times greater than among adolescent males[7], a gap that is reported in many other countries around the world including Brazil and South Africa

*[1]UNAIDS*
*[2]Karen Stanecki plenary address "Doing the right thing" XV International AIDS Conference, 11 July 2004*
*[3]International Conference on Women and Infectious Diseases 2004 (Atlanta, Georgia)*
*[4]Zeda Rosenberg plenary address on microbicides XV International AIDS Conference, 15 July 2004*
*[5]UNAIDS*
*[6]US Centers for Disease Control and Prevention*
*[7]UNAIDS 2004 Report on the Global Epidemic*

*continued from page 1*

Bangkok meeting touched on these issues and they were explored in greater depth at the Microbicides 2004 (M2004) in London in March. A meeting of funders, researchers and advocates was also held in April in Washington, DC that laid the groundwork for an over-arching coordinating body that may bear some resemblance to the nascent HIV Vaccine Enterprise.

### *Preparing for—and debating—efficacy trials*

By the end of 2005 as many as five efficacy trials involving six candidate microbicides could be underway (see Table 1). Preparation for these trials has engendered creative and provocative tensions as the field has re-assessed the current candidates in light of more recent scientific techniques and understanding. Five of the candidates come from the same class (polyanions); the sixth candidate, SAVVY or C31G, is from the same class (surfactant) as nonoxynol-9 (N-9), the spermicide that failed to show efficacy—and even appeared to potentiate HIV infection in some groups—in a Phase III trial completed in 2000. Pointing to the similarity among the candidates, and concerns about the general safety profile of surfactants, critics within the field have suggested that not all of the trials should go forward.

"The majority view is that the five trials scheduled to begin should go ahead essentially as planned," states Alan Stone, who chairs the International Working Group on Microbicides. "The minority view is that the case for going ahead with all six products is weak and that some of them should be put on ice."

The current debates are informed by recent studies that have applied newer scientific techniques to these first generation candidates, many of which were developed more than a decade ago. For example, most of the candidates scheduled for testing were evaluated in macaque challenge models using X4-tropic strains (which use CXCR4 as coreceptor) of SHIV, even though R5-tropic viruses (which use CCR5) are thought to be responsible for the majority of sexual infections. (A lab-adapted R5-tropic SHIV has recently been developed and is being used in some animal model evaluations of newer microbicide candidates.) In a plenary address at the M2004 conference, Robin Shattock (St George's Hospital Medical School, London) reviewed data suggesting that polyanions may be less effective against R5-tropic strains. This is because polyanion activity is mediated through an interaction with the positively charged V3 loop; this region is exposed on unbound X4-tropic viruses but only becomes accessible on R5-tropic viruses during cell binding and fusion.

Shattock suggested that the next generation of products should reflect newly-acquired scientific insights. "We now need to move into a stage of rational drug development, especially now that we know more about target cells." Shattock also suggested that pre-clinical development incorporate more studies of tissue penetration, since products which work by blocking cell-virus interactions will have to cross the vaginal mucosa and perhaps reach the draining lymph nodes to be truly effective.

Rather than arguing against any of the trials, many leaders in the field are instead urging that the upcoming studies be carefully designed—and supplemented with coordinated pre-clinical testing using newer methods. "It's really important to move forward with these Phase III trials; they will certainly help validate pre-clinical assays and we're hopeful that the candidates will show some efficacy. Even a partially effective candidate could be used in combination," says Rosenberg.

Stone too is a proponent of moving ahead with the planned trials, but says that

## Table 1: PLANNED OR ONGOING MICROBICIDE EFFICACY TRIALS: 2004-2005

| Product (class) | Primary research group | Proposed start date | Sites & sample size | Trial details |
|---|---|---|---|---|
| BufferGel (acid buffer)<br><br>PRO 2000 0.5% (polyanion) | HIV Prevention Trials Network | September 2004 | 3,100 women at 10 sites in Malawi (2), South Africa (2), Zimbabwe (2), Zambia (1), Tanzania (1), India (1), USA (1) | Four-arm Phase IIb trial comparing two candidates with two control arms (placebo gel and condom-only arm); includes Phase II safety trial run-in |
| Cellulose sulfate (polyanion) | Global Microbicide Project | Q4 2004 | 2,574 women at 6 sites (Benin, Burkina Faso, Kenya, India, South Africa) | Two-arm Phase III trial |
| Cellulose sulfate (polyanion) | Family Health International and Global Microbicide Project | June 2004 | 2,160 women at 2 sites in Nigeria | Two-arm Phase III trial |
| Carraguard (polyanion) | Population Council | March 2004 | 6,300 women in South Africa | Two-arm Phase III trial |
| PRO 2000 2% (polyanion)<br><br>Dextrin-2-sulfate (polyanion) | UK Microbicides Development Programme | Q1 2005 | ~12,300 women in Uganda, Zambia, and Tanzania | Three-arm Phase III trial comparing two candidates with placebo gel control arm |
| SAVVY (surfactant) | Family Health International | March 2004 | 2,142 women, 2 studies combined at sites in Nigeria and Ghana | Two-arm Phase III trial |

*Three of the candidates are being tested in more than one efficacy trial, in part because of recent FDA guidance stating that it would consider licensing a microbicide on the basis of efficacy data from one trial with a p value of 0.001 or two trials with p values of 0.005.*

"it makes a lot of sense for the planned trials to be organized so as to maximize the potential for comparative safety, effectiveness and acceptability." Stone and others have suggested linkages or cross-membership among the Data Safety and Monitoring Boards for each of these trials, so that class-specific similarities and differences can be more readily and rapidly detected during the course of the trials.

In the weeks following the M2004 conference, a group of funders, researchers and advocates involved in microbicide research discussed opportunities for coordination at a meeting convened by the Bill & Melinda Gates Foundation and the Alliance for Microbicide Development (AMD). The group recommended forming a coordinating body that would help facilitate harmonization across a number of areas including protocol design, monitoring, and decision-making for next-generation candidates. At press time, an initial proposal for such a body had been reviewed by major funders in the field and broadly approved for prompt implementation. "These discussions wouldn't have happened without the debate [over efficacy trials]," says Polly Harrison, executive director of the AMD.

### *Multiple pathways, multiple targets*

The microbicide candidates currently entering clinical trials are broad-spectrum candidates. The next generation of products is likely to emphasize more targeted approaches and to exploit new insights into the earliest events of sexual transmission.

The current understanding of this complex process was described in detail in plenary talks at M2004 by Shattock and Melissa Pope (Population Council, New York). As both speakers described, the vaginal mucosa contains several cell types that are susceptible to HIV infection, including $CD4^+$ T cells, dendritic cells (DCs) and macrophages. HIV uses a variety of co-receptors to enter these cells, including CD4 and CCR5 for T cells. In addition, HIV can also utilize C-type lectin receptors, including DC SIGN, mannose receptor and Langerin, to attach to or enter DCs and macrophages (see Review, page 1). Having picked up virus, migratory DCs can ferry it to the draining lymph nodes where it can infect many more $CD4^+$ T cells as a result of the normal DC-T cell interactions.

An effective microbicide could work by preventing the virus from reaching its target cells, perhaps by serving as a physical barrier in the mucosa or through virucidal activity in the vaginal lumen; however, it is highly likely that a truly protective candidate will also have to block some or all of these virus-cell interactions to prevent establishment of local and/or disseminated infection.

A presentation by Qinxue Hu (St

George's Hospital, London) gave some indication of the complexity of designing such a candidate. Hu reviewed a series of studies (*J. Exp. Med.* **199**, 1065; 2004) that tested the ability of various agents to block R5-, X4- and R5X4-tropic HIV infection in cervical explant models and DC cell lines. Compounds tested included AOP RANTES (CCR5- and CCR3-inhibitor), AMD3100 (CCR5-inhibitor), TAK-779 (CCR5-inhibitor), mannan (mannose receptor blocker), monoclonal antibody (mAb) RPA-T4 (anti-CD4), and mAb b12 and the fusion protein CD4-IgG2, both of which target gp120.

One study in activated (phytohemagglutinin-stimulated) cervical explant tissue found that AMD3100 and TAK-779 together inhibited infection by an R5X4 tropic isolate by about 80% as measured by p24 antigen release, emphasizing that CCR5 and CXCR4 are the primary co-receptors involved in infection of human cervical tissue.

The researchers then asked whether the panel of inhibitors could prevent DCs from internalizing whole HIV. They collected migratory cells emigrating from activated cervical explants 48 hours after HIV inoculation in the presence or absence of inhibitors, and then co-cultured them with indicator cells (PM1) to determine whether they contained infectious virus. Migratory cells containing infectious virus could be found in samples that were exposed to TAK-779 and AMD3100, suggesting that compounds which only block co-receptors may provide incomplete protection and that infection via migratory cells might still proceed. Substantial (80%) inhibition of infection of migratory cells was only achieved by a combination of mAb RPA-T4 and mannan.

Is it possible to simultaneously block the pathways that lead to localized infection and viral dissemination? As Hu reported, the only substances capable of blocking both pathways were ones that targeted gp120, rather than cellular co-receptors. MAb b12 and CD4-IgG2 reduced infection of T cells and migratory DCs by more than 95% in activated cervical explant tissue.

Extrapolating to vaccines, study co-author Shattock says, "It's a really striking thing that if you do have neutralizing antibody, it just halts infection—even neutralizing virus that is taken up by DCs."

**weeks ago, I could not have predicted that this would be a watershed conference for microbicides**

**Zeda Rosenberg**

### *Assessing the impact of local immune activation and inflammation*

Heterosexual transmission accounts for the majority of new HIV infections worldwide, yet relatively little is known about the determinants of male-to-female or female-to-male viral transmission. This is due to the difficulty of identifying individuals during the acute phase of infection and to the limitations of current methods for measuring mucosal immune responses. The available data strongly suggest that sexually transmitted infections (STIs) and bacterial infections such as bacterial vaginosis in women and urethritis in men can all increase viral shedding in the genital tract of HIV-infected individuals. These infections—particularly herpes simplex virus type 2—may also increase susceptibility to HIV infection in men and women.

Several mechanisms may explain these enhancing effects, including STI-related ulcers which can serve as portals for viral entry across the mucosa. Non-ulcerative infections have also been linked to increased susceptibility to HIV infection and it's thought that local immune activation may play a role, perhaps by triggering proinflammatory responses that enhance viral replication or by increasing the number of activated $CD4^+$ T cells and DCs trafficking from the genital mucosa to the lymph nodes.

STIs and bacterial infections that enhance viral replication might raise the bar for microbicide or vaccine efficacy. Many planned efficacy trials (see Table 1) and vaccine preparedness efforts are collecting data on STI prevalence and incidence in order to tease out the impact of STI coinfection on other interventions; diagnosis and treatment of STIs is considered an important HIV prevention strategy. By the same token, a microbicide that protected against STIs other than HIV might still have a protective effect against HIV simply by reducing inflammation.

Other agents may actually cause inflammation. Like all mucosal surfaces, the vaginal epithelium can respond to any foreign substances, including microbicides and lubricants. At M2004, a presentation by Gustavo Doncel (CONRAD, US) showed that changes in the local vaginal environment can be caused by vaginal products such as lubricants, spermicides, placebo gels and candidate microbicides. Doncel measured the expression of proinflammatory genes and cytokines, including interleukin (IL)-1, -6, and -8 in immortalized human vaginal keratinocytes (VK-2/E6E7) that were pre-treated with candidate microbicides, placebo gels and over-the-counter vaginal lubricants. Some of these products, including N-9 and a lubricant, caused increased production of these cytokines and upregulation of NF-κB.

Doncel is working to improve the field's ability to identify candidates with potential safety problems at very early stages in the development process, a consequence of the adverse effects reported for N-9 during the Phase III efficacy trial. The standard preclinical safety analyses for that trial included *in vitro* cytotoxicity studies and a standard panel of animal safety tests that failed to predict the observed effects. But later analyses found that N-9 use increased proinflammatory responses, including cytokine and chemokine induction and an influx of activated macrophages in the cervicovaginal lavage (CVL) (*J. Infect. Dis.* **184**, 418; 2001). An assay such as Doncel's that measured pro-inflammatory responses could be used as an early indicator of product safety, and several presenters suggested that these data should be collected for the candidates currently entering Phase IIb and Phase III trials. Efficacy data could then later be compared with these results to help validate predictive assays.

The field is also seeking early indications of product efficacy that could help guide decisions about launching large-scale efficacy trials. At M2004, Marla Keller (Mount Sinai School of Medicine, New York) described a strategy for gathering CVL fluid from HIV-infected women before and after treatment with a candidate microbicide called PRO 2000. Diluted CVL was spiked with HIV and inoculated

on to susceptible cells to measure viral replication. Preliminary results indicate that post-treatment CVL inhibited replication of both HSV-2 and HIV. Since women already infected with HIV but unaware of their status will inevitably use any effective microbicide developed, safety and efficacy—including effects on viral activity and shedding—must be evaluated in this group. The same type of study could be used to gather preliminary data in HIV-uninfected women.

### *Timing of early events in sexual transmission*

As the picture of sexual transmission becomes clearer and more detailed, both vaccine and microbicide researchers are focusing on the timing and sequence of early events—specifically the interval between initial infection of target cells in the vaginal mucosa and dissemination to the draining lymph nodes. Rapid dissemination would pose challenges for both of these prevention measures, since rapid transport to the draining lymph nodes could remove HIV from either sufficiently inhibitory concentrations of microbicide or potentially protective vaccine-induced immune responses.

Under some conditions viral dissemination may occur within a matter of hours. Chris Miller (University of California, Davis) and colleagues found SIV-infected cells in the draining lymph nodes within 18 hours of intravaginal exposure to highly-pathogenic SIVmac251 (*J. Virol.* **74**;6087, 2000). However there may also be conditions under which systemic infection does not emerge for days or even weeks after initial exposure. At M2004 Miller raised this possibility with data from a new study (in press, *J. Virol.*) in which eight macaques were challenged intravaginally with multiple low-dose inocula ($10^3$ $TCID_{50}$ of SIVmac251) over the course of several weeks; the animals became infected after an average of 8 challenges. In six infected animals, systemic infection was preceded by a period of transient viremia followed by an interval of "occult infection," during which no virus was detected in PBMCs. Some of these animals also had SIV-specific T-cell responses during the period of occult infection.

Miller noted that these results differ from the kinetics of viremia and immune responses in animals infected with a high dose intravaginal challenge, after which systemic infection develops immediately with subsequent emergence of SIV-specific immune responses. He suggested that timing of viral dissemination may vary depending on the size of the initial challenge and that, under certain conditions, multiple exposures (such as might occur during intercourse with an infected partner) might give rise to localized infection that is not detectable in the blood. Miller also referenced the highly exposed, persistently seronegative sex workers in Nairobi, some of whom have HIV-specific immune responses in the absence of detectable HIV infection. He suggested that some of these women might actually have occult infection and that this phenomenon might be more common than is appreciated.

The establishment of infection at the portal of entry and timing of dissemination might also be affected by the numbers and types of cells that are initially infected. In a paper published shortly after M2004 (*PNAS* **101**, 5640; 2004), Ashley Haase (University of Minnesota) proposed that HIV exploits whichever cell types are most common in the vaginal epithelium, draining lymph nodes and other lymphatic tissues to which virus spreads. These are principally resting T cells, which have not been considered permissive targets for HIV infection, but Haase found that small Ki67 negative T cells—which he thinks might actually be activated cells returning to a resting state—can indeed be infected. (Ki67 is a marker of cell proliferation.) In the healthy mucosa, these cells greatly outnumber activated T cells, DCs and macrophages, and are thus more like to be infected because of their availability. While they only maintain a low level of viral replication, Haase thinks they may play a critical role in sustaining infection in its earliest stages and that the role that the infected activated $CD4^+$ T cell plays is to more efficiently disseminate virus because of higher levels of replication. "The kinetics of infection will depend on the sizes of these founder populations of infected cells, and in turn on factors that influence the integrity of the mucosal barrier and thus the access of virus to cellular targets" Haase says.

A novel *ex vivo* model presented by Julie McElrath (University of Washington) could shed additional light on the types of cells first infected. McElrath described a model developed with colleague Florian Hladik that uses a suction blister method to separate the outermost epithelial layer from the underlying stroma of sheets of vaginal epithelia obtained from healthy women undergoing tissue repair surgery. She described how the basal side of the epithelium remains fully intact during the procedure, allowing for *in situ* analysis of virus-cell interactions with minimal damage to or changes in cell function. The basal side of this epithelial sheet displays an organized pattern of deep depressions which nearly reach the vaginal surface. Most of the mucosa's potential target cells for HIV (infiltrating lymphocytes and Langerhans cells) accumulate around these basal depressions, which are also among the thinnest regions of the epithelial barrier and therefore most easily breached.

McElrath presented unpublished data from a study in which sheets of the outer epithelial layer were co-cultured with fluorescently-tagged lab-adapted strains of HIV. Confocal and electron microscopy of fixed sheets revealed HIV on the surface and inside of both $CD4^+$ T cells and Langerhans cells, suggesting that the two cell types might be infected in parallel. (One model of HIV infection holds that DCs are the first cells to be infected and that they then transmit the virus to $CD4^+$ T cells). In discussion, McElrath noted that since her group had not yet seen viral budding or fusion from epithelial cells, it was not possible to say with certainty whether HIV productively infects these cells or merely sequesters virus within these cells. This new model remains to be fully validated, but it could prove to be an additional tool for understanding early events in a physiologically relevant system.

### *Next steps*

The next microbicide biannual meeting will take place in 2006, as will the next International AIDS Conference. By then early data could be available on safety or perhaps even efficacy from the microbicide candidates currently advancing into clinical trials. These data will give the first hint of whether these current approaches provide any protection against HIV infection. As new tools and techniques are developed, the next two years will likely bring further insights into the early events of sexual transmission of HIV that will help guide the design of next generation microbicide and vaccine candidates. The challenges are formidable, but as Zeda Rosenberg said in Bangkok, "The science is there, the technology is there, and most of all the passion and dedication of those in the field is palpable. And failure is not an option."

Perspective

# Microbicides come of age?

**by Robin J Shattock PhD**

Growing up can be a painful business, especially when the optimism of youth is tempered by the reality of experience. Microbicides, often seen as the lesser sibling of vaccines, therapeutics and safe sex promotion, are finally coming of age: six products are currently moving into large Phase III trials with a pipeline full of new candidates following close behind. What should this field learn from its older relatives, and how best can microbicides be embraced as an ally in the fight against AIDS? The next few years will be critical in seeing how the family dynamics play out. The promise of microbicides is that they could be available within a far shorter timescale than an effective vaccine or wide-scale treatment. But how realistic is such tantalizing promise, and what hurdles exist to realizing early gains in HIV prevention?

Microbicides, vaginal formulations designed to prevent transmission of sexually transmitted infections (STIs), and more recently specifically HIV, are by no means a new idea. Topical agents from lemon juice to soap have been applied by women for generations in an attempt to counter STIs. But early hopes that simple agents capable of destroying viral particles would provide a rapid solution to the HIV problem were dashed when it was observed that the surfactant candidate nonoxynol-9 not only failed to prevent infection but increased susceptibility amongst frequent users of the product. Experience has taught that, above all else, microbicides must not disturb natural physical barriers to infection. Thus, as with vaccines, the microbicide field has had to accept that HIV protection may be more complex than first thought.

Meanwhile, in the early 80s several independent groups started work on developing polyanion-based microbicides that, instead of destroying viral particles, interfere with processes of viral attachment and fusion with target cells. Starved of funding in a climate that expected imminent development of an effective vaccine, progress was only sustained by a small but dedicated field of researchers and supportive project officers. This important but uncoordinated effort led to parallel development of similar products. More recent acceptance that an effective vaccine was still far from being realized, that safe-sex promotion was failing to halt the epidemic and that treatment for all might not be attained any time soon, has led to a surge in microbicide funding. The net result: five products with similar modes of action (polyanions) and one surfactant based product (SAVVY) all entering Phase III clinical trails. Whether agreement with the rationale for taking all six products into large-scale efficacy trials is a “majority” or “minority” viewpoint depends on who you talk to. While funders grapple with conflicting allegiances (see article, page 1) and intended trial sites stand vacant, many scientist struggle with the scientific rationale for such an approach[1], noting uncomfortable parallels with the vaccine field[2].

In contrast, rapid developments in understanding the mechanisms of HIV transmission (see Review, page 1) is bringing a new appreciation to both vaccine and microbicide development. The large number of different cellular receptors involved in establishing HIV infection and the rapid kinetics of viral dissemination within an infected individual suggest that no single approach may be effective[3].While for vaccines this may mean harnessing innate, cellular and humoral arms of the immune response, for microbicides it may mean targeting alternative and/or sequential pathways involved in mucosal infection and rapid dissemination to draining lymph nodes; in particular viral attachment, fusion and proviral formation[4]. Time and distribution of viral exposure present further challenges for any intervention strategy; HIV infection of susceptible cells within mucosal tissue can occur within minutes, while dendritic cell uptake of the virus may maintain its infectivity in mucosal tissue for several days. For vaccines this may mean that sufficiently high levels of specific effector cells (B and T cells) may have to be maintained in order to prevent infection. For microbicides it means that compounds targeted against infectious virions and/or infected cells must be adequately distributed within the genital tract at concentrations sufficient to neutralize or inactivate virus within minutes. In contrast, compounds (e.g., chemokine antagonists, fusion inhibitors, or reverse transcriptase [RT] inhibitors) targeted against susceptible cells must be able to reach their specific targets (e.g., dendritic cells, macrophages, and T cells) at least as well as the infectious virus and may need to be present for prolonged periods. Thus it is highly likely that a combination of such approaches may be required for an effective HIV microbicide. While some combinations may demonstrate synergistic anti-HIV activity, others may be required to provide simultaneous blockade of multiple transmission pathways. In this respect microbicides could learn from its other older sibling, HIV therapeutics, where combinations are critical for viral control. Furthermore, resistance to some microbicide candidates may also occur if used by women unaware of their HIV status. The likely use of RT inhibitors (e.g., nucleoside inhibitor PMPA and non-nucleoside inhibitors like TMC120 and UC781) as microbicide candidates means this lesson may have particular relevance to both fields (microbicides and treatment) since resistance induced by either approach would compromise the efficacy of the other.

> ***a partially-effective microbicide might show significant synergy with a partially-effective vaccine***

Clearly there is a strong scientific rationale for rapid development of combination microbicides and such an approach will ultimately provide the best chance for demonstrating efficacy in clinical trials. So

what are the hurdles to rapidly moving combinations into efficacy trials? The first derives from a reluctance to share intellectual property rights, or a desire to demonstrate efficacy for "own" or "owned" agents before considering combinations. Yet the whole objective of microbicides—to provide cheap, affordable protection—doesn't equate with large profits. Pharmaceutical companies need to join the field, not with an eye to the bottom line[5] but for the possible PR value: not an impossible dream when you see the landmark agreement signed between Tibotec (a subsidiary of Johnson and Johnson) and the International Partnership for Microbicides[6]. Other constraints include a less than clear pathway to regulatory approval, although approval of an effective combination may ultimately be easier than for a merely partially-effective single agent. But perhaps the biggest hurdle to rapid combination development is the sequential nature of clinical trials. While the current planned Phase III trials may have gone beyond the point of no return, it would seem almost reckless to persist in a pattern of linear, sequential design of single-agent trials of products with similar activity when combination products can be justified, conceptualized, and tested now. For effective microbicides to be realized in a meaningful timescale, the field as a whole and funders in particular need to be able to prioritize candidate and combination selection and provide a fast track into clinical efficacy trials. Such vision and co-ordination has yet to emerge.

There is another promising relationship that to date has been unexplored: the potential synergy between vaccines and microbicides. Traditionally this has been discussed in terms of shared trial sites, placebo arms and trial infrastructure. It is unclear whether a true synergy of effort can take place by performing different prevention trials in the same sites or whether possible crossover between study participants would obscure data analysis and/or saturate recruitment capacity. However, an alternative synergy between these fields may also exist. A partially-effective microbicide might show significant synergy with a partially-effective vaccine, the former significantly lowering the viral challenge with which any vaccine-induced immune response needs to contend. Furthermore, protective immunity to HIV is likely to require prolonged raised mucosal immune responses, yet such responses are typically short-lived. Although controversial, some have suggested that studies in cohorts of highly-exposed persistently seronegative (HEPS) women means that repeated vaginal exposure to antigen may be required to maintain resistance to infection. Microbicides could be used to deliver relevant HIV vaccine antigens recurrently, maintaining mucosal immunity induced by conventional vaccine approaches. Likewise, any resultant enhancement of mucosal immunity would augment microbicide efficacy where there was suboptimal compliance. Although such synergy is at present mere conjecture, the potential benefits warrant further investigation.

Finally microbicides could also learn from one other older sibling, the field of safe-sex promotion. Despite renewed emphasis on "ABC" programs (abstinence, be faithful or condomize), this strategy is clearly failing women for it implies they always have a choice. The sad reality for most women at risk is that they often cannot choose abstinence, that faithfulness only works when adopted by both partners, and that condom use is predominantly controlled by men. This is all the more pertinent in stable relationships where condom use becomes an issue of trust and the fear of being barren is worse than that of becoming infected with HIV[7]. Ultimately the success of "ABC" is determined by the actions of men. Microbicides, in contrast, are aimed at empowering women since they may be applied covertly without their partner's knowledge. Yet too much emphasis on their covert use could have a negative impact. By implying a level of suspicion about a partner's faithfulness, women may be afraid to use such products for fear of being found out: and if the product is easy to disguise, there are still the applicators to hide. There is also the issue of timing—appropriate application of a microbicide would require a degree of anticipation that is often not available to many women. So microbicides are most likely to succeed if they have both sustained activity, allowing them to be applied hours (perhaps days) before intercourse, and male approval. But male approval could also have negative connotations if introduction of a partially-effective microbicide led to migration away from condom use. While this specter has often been raised, recent modelling has predicted that for a microbicide with only 50% efficacy to have a detrimental impact on transmission rates, condom use would have to be higher than 80% (ref 8).

So how might the future play out? If lessons from the past are ignored, the future might look something like this: Lack of prioritization and insufficient funding (currently still a fraction of the budget for other prevention measures) means effective microbicides are not developed for a decade or more. Clinical trials again demonstrate little to no efficacy and/or adverse effects. Developing countries and potential donors become less willing to support microbicide efficacy trials (the same might also be true with repeated vaccine failures). Competition for sites with the vaccine field means that trials become harder to perform. Early introduction of partially effective microbicides leads to decreased condom use and even antiretroviral resistance.

However, if the field has the vision to learn from previous experience, a very different future might be realized: Rational prioritization of candidates and combinations provides a fast track into human efficacy trials. First generation products show detectable and significant efficacy (>30%), second generation products, most likely combinations, demonstrate increased efficacy (>70%). Subsequent introduction of sustained release formulations improve subject compliance leading to increased gains in efficacy. Joint condom and microbicide promotion a success; some reduction in condom use, but benefits of microbicide use in unprotected sex acts leads to reduction in transmission rates. Microbicides demonstrate potent synergy with partially-effective vaccines and/or are formulated to maintain mucosal immunity. Finally, microbicides are strategically marketed so that they become a desirable commodity, creating a sustained demand for product and ongoing use.

It is easy for a young field to be full of promise, but growing up means being able to deliver. The future that microbicides deliver may be as dependent upon the ability to profit from the past as it is on a clear decision path for the future.

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An Interview with

# Susan Allen

# Serodiscordant Couples: Africa's Largest HIV At-Risk Group

***Susan Allen has been a lead investigator of the HIV epidemic in Africa, particularly as a pioneer in the study of HIV transmission between couples, the primary way in which HIV is spread in Africa. She established Project San Francisco in Kigali, Rwanda in 1986, one of the earliest and now longest-standing HIV cohort studies in Africa. In 1994, as a result of the genocide in Rwanda, Allen relocated the central research clinic to Lusaka, Zambia (the Zambia-Emory HIV Research Project), which has recruited the largest single-site heterosexual HIV-discordant couples (in which one partner is HIV-infected and the other is not) cohort in the world. The unified sites now employ over 150 healthcare staff and are known together as the Rwanda Zambia HIV Research Group (RZHRG); they continue to enroll and follow HIV-discordant couples.***

***Allen, who has MD, MPH and Diploma of Tropical Medicine and Hygiene degrees, has just taken up a new position as Professor of International Health at Emory University's Rollins School of Public Health. Her mission is driven by the recognition that the largest at-risk group for HIV in Africa—couples—constitutes a public health crisis. Not merely a research endeavor, the project sites operate voluntary counseling and testing (VCT) centers that test and counsel couples together, providing HIV/AIDS education, free HIV testing and counseling surrounding test results, and provide free outpatient reproductive healthcare and treatment of infectious diseases for eligible study participants. Such counseling has had quantifiable results: post-counseling HIV incidence rates have been 50-70% lower than those of non-counseled discordant couples. "The principal research objectives of the RZHRG are to uncover factors relating to HIV risk and transmission, to gain a better understanding of how to prevent the spread of HIV and to improve the quality of life among HIV-infected African adults" explains Allen. She recently spoke with* IAVI Report *editor Simon Noble about HIV/AIDS in Africa and the diverse activities at her project sites there.***

***You've pioneered recruiting cohorts of co-habiting couples. What makes such cohorts particularly valuable?***

Well, I think it's by far the best way to identify suitable cohorts for vaccine trials because, at least in Africa, the largest group at risk is couples. What few people seem to remember is that 60 or 70% of the transmissions that happen everyday in Africa occur between spouses, and so lots of people focus on higher-risk groups, like sex workers or truck drivers, and while those are certainly worthy of study they're not the largest at-risk group that account for most of the transmissions. The biology of transmission is likely to differ by both route and inoculum size, so if you were going to develop a vaccine that would work against blood-borne transmission for intravenous drug users, it might have to have different characteristics to one that would protect against a mucosal challenge. And if you were developing a vaccine that was going to protect against a mucosal challenge when the mucosa is really impaired, like in a sex worker who has lots of different partners, lots of trauma and lots of inflammation, again that's a different scenario biologically than a husband and wife.

Once we find a [vaccine] product that works we are going to start vaccinating large groups of people, so the largest at-risk groups are the ones that we should study the vaccines in; we should be doing trials in discordant couples, [which are] cohabiting couples where one partner has HIV and the other doesn't.

***How do you go about recruiting these couple cohorts?***

The mechanics are that you have to go out and promote couples VCT (voluntary counseling and testing), which is catching on among funding agencies and governments but is not yet established as a norm in the population. There's this awkward two-legged stool; certain groups, like pregnant women, are being targeted for VCT for prevention of transmission to their babies, but most pregnant women are married and it would be so easy when you're testing them to include the husband. But the men are left out, and the capacities of a lot of ante-natal clinics don't really allow male participation unless they have weekend programs.

We've piloted a couple of programs but it requires a concerted effort and so far those worlds, the couples VCT and the prevention of transmission from mother to infant, haven't met. So the best way we've found to do it is to go out in the community and promote couples VCT and then provide the services to couples coming in. We have our own services—our facilities are stand-alone NGO-run—and so we don't over-burden government facilities.

***What kind of new strategies can be employed to get more men enrolled in VCT programs?***

Any time VCT is being offered by a health-care provider, for whatever reason—whether it's a blood donor, a sick person seeking hospital care, a TB patient, a pregnant woman—the provider has to think who might be married, which is the case in Africa for most adults; certainly most women over the age of 22, 23 are married, most men by the time they're 29 to 30. It's just a question of having the people who provide those services automatically and reflexively think, "And your partner?"

***Do you think that vaccine efficacy trials will be a possibility in couple cohorts? Is the power there to get the relevant numbers?***

Oh yes, absolutely. In fact, I think they're the best cohort because the partners support one another. Even in discordant couples, the majority of them are committed to each other and they don't separate. They do begin to use condoms after they learn their HIV status, certainly more than they did before, going from something like 0 to 90% use at blinding speed. But they don't use them perfectly so there continues to be transmission at a relatively high rate, about six to eight percent a year.

In the past when researchers first started looking at couple cohorts and the couples didn't know they were discordant—and this is from cohorts that I wasn't involved with—the transmission rate was about 20 or 25% a year. So you can see that just knowing has a huge impact. But you're left with this residual rate of six to eight percent, which is still high, so compared to other cohorts that could be used in vaccine trials, discordant couples are still definitely contenders. The other really key thing is that when a transmission event does happen in a vaccine trial, you have the donor whose virus you can study as well.

Depending on where you go, the proportion of the couples that you test that are discordant will differ. In Lusaka [Zambia], for example, our couples VCT centers test lots of couples and 20% of those will have one HIV-positive and one HIV-negative partner. In Kigali [Rwanda], the proportion is 10%.

***Do you think discordant couples are a feasible way to specifically increase the number of women participating in trials?***

Yes, that's actually what we're trying to do, both in Rwanda and Zambia, so that there's a gender balance in trials, that there are half men, half women.

***In Zambia and Rwanda specifically, what are the challenges to recruiting women to participate in trials?***

The tricky part with women is that they can't be pregnant or breast-feeding to be in the trials. There's lots of willingness [to participate], and since our discordant couples include women who are HIV-negative and who have HIV-positive partners and vice versa, we can recruit from among discordant couples and have the vaccinee be either the man or the woman, depending on who the negative partner is. But the HIV-negative women have the added challenge of needing to be not pregnant or breastfeeding, so at intake you have to ask whether they would be willing to use a long-acting method of contraception, like IUD or hormonal methods, to avoid conceiving in the 12 to 18 months that they're in the trial. Of course, these would be supplementary to the barrier methods, male and female condoms, that they need to use to prevent HIV infection.

***Is there any good evidence that hormonal contraceptives might have an effect on transmission susceptibility?***

Not in our cohorts. In fact, if anything, it's protective for progression of disease. In the HIV-positive women in Rwanda that we have followed for 18 years now, use of injectable or oral hormonal contraceptives was associated with increased survival. And, among the HIV-negative women, use of those methods was not associated with increased acquisition [of HIV]. Now, I know different results have been found in sex workers but, again, the whole ecology of the genital tract in sex workers is completely different.

***In some communities in Zambia, your work has established that marriage is a risk factor for women becoming infected with HIV. What can be done to reduce their susceptibility to infection?***

The only way to do it at the moment is couples testing and counseling. If you can get it moved into the premarital realm, which is part of what we're working with the churches to do, that's the best way to do it, and premarital testing is actually becoming more of a

**the largest at-risk groups are the ones that we should study the vaccines in; we should be doing trials in discordant couples**

social norm than a lot of people realize. But if it isn't done premaritally then it needs to be done amongst married couples.

***Do you think there is sufficient awareness in some developed countries of the phenomenon of marriage as a risk factor?***

No. I think a big part of what we're trying to do with our educational campaigns is to make people aware that just because you're married to someone doesn't mean you're protected. A lot of the prevention messages in the early days would say things like, "Stay faithful and you'll be okay," but if the person you're faithful to has HIV and you don't then that's not going to help you. That's the big hurdle to overcome, in particular because women tend to have fewer partners outside of marriage than men. So if women believe, "Okay, if I'm faithful to my husband, I'll be okay," that's often not the case.

***What's been your experience in providing comprehensive family planning services in the context of HIV prevention, especially with the recent calls from some quarters for the promotion of the 'ABC' [Abstinence, Be faithful, Condomize] programs?***

We've done a lot of research on combining family planning messages and HIV prevention messages in married couples, and I have to say that in married couples we set the whole 'A' aside, abstinence is not even on the radar in married couples. And no church would recommend it. But in terms of deciding whether to use barrier methods and/or other types of contraceptives, we promote the dual-method message—female or male condoms as a barrier method are good for the prevention of HIV and other STDs, but they're not great contraceptives. So in our counseling we recommend having added protection against pregnancy, adding IUD or Norplant or an effective long-acting method in addition to the barrier method that they use for prevention of HIV/STD.

***Given that in many societies men have disproportionate power in sexual relations, what is the most effective way to increase condom use?***

We find that the key is having the husband and wife, or cohabiting partners, however you define the marriage, do the pre- and post-HIV test counseling together. If they get their test results in the room together and they make a plan based on the combination of their test results, then condom use is optimized. We've measured that in all kinds of objective and subjective ways.

***What have been the specific concerns of community advisory boards (CABs) with regard to your couple cohorts? Are there ethical dilemmas specific to couple cohorts and their counseling? Particularly in discordant couples, I would imagine.***

Our CABs have always been really supportive, I think because they realize that once you have a couple that's been tested and counseled, and they know that one has HIV and the other doesn't, you've done the very best you can in terms of counseling and support, promoting condom use, providing ongoing follow-up and so forth. And, you know, people are human, they don't always do what's best for them all the time, so it's understood that whether it's because they might want to have a baby or some other reason, they might have sex without a condom or might not use a condom correctly occasionally. That's certainly the case for most discordant couples, there continues to be risk.

**while you try to do the very best you can with counseling… you're never going to have complete protection with that approach**

So the CABs understand that while you try to do the very best you can with counseling and you try to continually improve your counseling, you're never going to have complete protection with that approach. It's a little like seat belts—you can legislate seat belts, and 80% of people will use them just to avoid getting a ticket, but 20% still won't, even though they know that it's life-saving and they know the accident statistics. You can only go so far with trying to get people to change their behavior. So CABs are very supportive of biomedical interventions, at least in our experience, once the rationale is understood.

***Are there any special challenges to counseling discordant couples? How does it differ from your regular VCT counseling?***

Well, you have three scenarios when you're giving results to couples. The first and happiest one is they're both negative, and that's obviously the easiest one because then you're trying to reinforce behaviors to keep them both negative, and there's a huge sense of relief and a renewed commitment to the relationship and faithfulness. Then you have a situation where both partners are positive, and that presents a challenge because they have to think about the kids they have and the fact that they might not live to raise those kids, and planning for their own health and the family's future and all kinds of things. But obviously there's less focus on transmission from one to the other because they both already have it.

With discordant couples, of course it's more complex, you have to support the HIV-positive person as an individual with HIV and their health issues and concerns, whether they're symptomatic or, more usually, asymptomatic. And then there's the issue of protecting the non-infected partner from acquiring HIV, they're more vulnerable because they're in that marriage. And if you have a discordant couple where the positive partner is the woman, then you have to deal with the added issue of, should she become pregnant, her child might acquire HIV.

We've taken an almost modular approach to counseling, there's the basic VCT and then you add things to it. If the woman's pregnant, that's another added message. If they're coming in together as a couple, that's another added message. It becomes a very complex business in the end.

***What kind of guidelines are in place to dictate how much information should be released about one partner's status to the other? For instance, one partner becomes HIV-infected, are you obliged to inform the other?***

In our couples VCT centers, we have the couples sign joint informed consent, and they see that informed consent by video. So for those that struggle to read, all the information is presented visually. So all the couples that come through our center sign saying they want to be tested and counseled together, they want to receive their results together, so we don't have a problem of disclosure between partners. But we do tend to advise people to be very careful before they choose to share the results outside the couple because they have to judge their own family members and how supportive they'll be.

When a seroconversion event happens—like in the course of our prospective studies, if we have discordant couples and at some point the originally negative partner becomes positive—at that point we counsel them about the fact that their serostatus has changed and what that all means.

***It strikes me, speaking to you now, that these marriages sound very strong. Is that something that's struck you?***

Yes, I think a lot of things go into marriages, and by the time people come through our doors, they've been cohabiting an average of six years. They almost all have children, some sort of ceremony has been held, whether it's a civil ceremony or traditional one, there's been a bride price exchanged. A whole community has been involved in that union, and so to separate, it's not done lightly. I would say Zambian and Rwandan urban couples tend to have the most erosion, if you will, of the traditional values, so in more rural areas I think you would find even more of that. So yes, I think married couples tend to stick it out, the whole for-better-or-worse thing. I have been struck, actually, by how committed husbands and wives are to each other in the face of this in Africa.

***So I understand that you're currently involved with IAVI in setting up the adeno-associated virus AIDS vaccine candidate Phase I trial in Zambia and South Africa.***

That's what we're hoping to do, we just had a consensus conference in Zambia which went well, and we're now going to submit the protocols for formal review to the ethics committee and the other regulatory committee which they call the Pharmacy and Poisons Board in Zambia. These trials follow on from the trials in Europe, in Belgium and Germany, and the proposed trials will test the immunogenicity and the optimal dosing schedule of the adeno-associated vaccine candidate.

***Could you talk about Project San Francisco, and how AIDS vaccines are being brought into that program?***

Project San Francisco is our program in Kigali, it's now 18 years old. We started out with pregnant women, like a lot of people do, and they said to us, "Would you test our husbands?" and it just sort of evolved—that interested us in couples and we realized that discordant couples were really the main risk group and should be our focus of interest. Then we had to temporarily leave Rwanda in '94 because of the genocide, and we set up an analogous project in Lusaka, which is now 10 years old, called the Zambia-Emory HIV Research Project, ZEHRP. The two projects together are under an umbrella that we call the Rwanda/Zambia HIV Research Group, RZHRG. We are staunch in our opposition to acronyms that mean anything. We're now working to set up AIDS vaccine trials under that umbrella group; the common theme between the two countries is the couple cohorts. The vaccine products that will be tested will probably be different because in Rwanda it's predominantly HIV clade A that's circulating, whereas in Zambia it's clade C, but the methodology and the risk groups will be the same.

***What other types of studies are coming out of these cohorts?***

Our two biggest focuses have always been prevention of transmission and the study of the natural history of disease; once somebody is infected, what happens? Eric Hunter, my husband, is studying the genetics of the virus and looking at the characteristics of the virus that is transmitted, or at least the virus that establishes infection, because these viruses are likely to be very important from a vaccine perspective. That work was published in *Science* a couple of months ago. Dick Kaslow and James Tang, who are immunogeneticists, have been looking at the genetics of the host. They've published a great deal on the genetic markers associated with living a long time once you have HIV, versus succumbing quickly to the infection, developing disease and dying. Just recently they had a paper in *The Lancet* looking at host genetics in HIV transmission in our Zambia discordant couples. The bottom line there is that the more similar husband and wife were with respect to the genetics that code for immune responses, the more likely it was that virus was transmitted from one to the other because being genetically similar means you have similar immune responses, at least with respect to the aspects that they studied.

We've also just done some behavioral studies this summer with a student from Emory, Joyce Au, where we're providing ARVs (antiretrovirals) to our sick patients in Rwanda with Global Fund support. We've just completed a survey of the partners in discordant couples who are sick and getting ARVs. Actually, that reminds me—in every case, their drug buddy was their spouse, who was helping them remember to take the drugs and so forth. We asked them, "So how has this affected your condom usage? Do you feel that you still need condoms? Are you okay now and you don't need them?" And 100% of the people that we interviewed said, "No, no, condoms are more important than ever because the last thing I'd want to do is transmit a resistant virus to my spouse." So they're extremely sophisticated in their thinking about this.

***What do you consider should be the key goals for HIV/AIDS prevention in the next couple of years, and perhaps in the more medium term?***

Well, applying behavioral strategies that we know work; in sub-Saharan Africa, couples testing and counseling. I think that should be a number-one promotional strategy, and CDC has adopted it as a centerpiece, even though they don't have much practical experience with it they have agreed that it's important. And to get more people on the bandwagon, that the largest risk group on the planet is cohabiting couples in sub-Saharan Africa, and the only thing that is known to work with them is couples testing. So that's number one.

And then the next step is vaccines, which I see as the most feasible biomedical prevention/intervention on the horizon, despite the obstacles.

***What kinds of things are you working on now and what do you think are the most exciting areas of HIV/AIDS prevention?***

Right now, my grants are behavioral in nature; promoting couples VCT, combining VCT messages or prevention messages with family-planning messages. Our cohorts act as a source of samples for studies like Eric's looking at the virus and Dick Kaslow's looking at the genetics of the host, and other investigators who are interested in immune responses. And then we're gearing up for vaccines, which is, I'd say, the most exciting thing we're doing right now.

Editorial

# Proof of concept

The recent announcement of the Phase II proof-of-concept efficacy trial of Merck's replication-defective adenovirus vector vaccine candidate, MRK-Ad5, is a very important step for the AIDS vaccine field. The collaboration between Merck and the HIV Vaccine Trials Network (HVTN) was announced in public discussions at the National Institutes of Health's AIDS Vaccine Research Working Group (see Vaccine Briefs, page 20) and then at the recent AIDS Vaccine 2004 Conference in Lausanne, Switzerland. The trial, scheduled to begin at the end of this year, will test one of the most promising AIDS vaccine candidates in development.

In ongoing Phase I and II trials, MRK-Ad5 has elicited in up to 75% of vaccinees the most robust HIV-specific cellular immune responses yet seen in humans. The new trial will determine if cell-mediated immunity, at least as currently defined, can be effective in either preventing HIV infection or at least reducing post-infection viral load; the latter would hopefully lead to improved prognosis for individuals and lowered transmission rates for populations. Results of the trial are expected to be available in late 2007 or early 2008.

This means the field has three years to prepare for success. Unfortunately, even if MRK-Ad5 proves to be an effective vaccine it will likely not be available for widespread use in humans. Adenovirus is a natural human pathogen that causes a manifestation of the common cold and pre-existing immunity to adenovirus serotype 5 (Ad5), on which the new candidate is based, is widespread in many human populations. In the US and Europe about 35% of the population has significant levels of immunity to Ad5 that appear to render the vaccine candidate ineffective at inducing HIV-specific responses; in many developing country populations that figure rises to more than 80%. A potentially precarious political aside to a successful proof-of-concept trial, therefore, will be to explain to the general public, particularly in developing countries, that an effective AIDS vaccine cannot be distributed widely. Trial sponsors would do well to get the point across early.

So between now and the end of the trial alternative adenoviral vectors need to be identified and evaluated. Pre-existing immunity to other human adenovirus serotypes seems to be far lower and some (like serotypes 11 and 35) are already being tested as vectors. Adenoviruses that naturally infect other species, chimpanzees in particular, are also being developed and assessed for their potential as immunogenic vectors. If several different adenoviral serotypes can be developed they might be used sequentially in prime-boost immunization regimens to increase the strength and durability of HIV-specific cellular responses (see Research Briefs, page 18).

These improvements and expansions of the adenoviral vector repertoire must be accompanied by efforts to ensure that the process development and manufacturing scalability potential are optimized, to ensure that once an effective vaccine is identified it can be produced in large quantities for distribution and use where it is needed most in the shortest timeframe.

In the same time period, the perennial problem of eliciting humoral immunity against HIV has to be cracked. Even if adenovirus vectors prove outstandingly effective, adding a broadly effective neutralizing antibody component to the vaccination regimen can only solidify protection.

Of course the field must also prepare for disappointment, and in such a scenario effective humoral immunity may prove even more crucial. As ever, novel vectors should continue to be identified and developed, perhaps ones that induce cellular immunity that is qualitatively different to that which is now induced. Those answers will come from further advances in basic immunology in many different systems, and HIV research must embrace and make partners of scientists in other disciplines. Advances in measuring and consequently understanding mucosal immunity will be important, as will insight into strategies to induce protective innate immunity. Better adjuvants and other innovative delivery strategies that will enhance the magnitude and duration of immune responses to practically useful levels are also needed.

The challenges remain as formidable as ever, so we must prepare for success but be equipped for a setback.

# New-look *IAVI Report*

We hope our readers will appreciate the newly re-designed *IAVI Report* which reflects our aspiration to become more of a forum for the AIDS vaccine community. In order to engage as many readers as possible and serve the research community, as we move forward we will strive to increase the 'harder' scientific content to encompass the latest research and be a forum for analysis and debate of the issues that will ultimately lead us closer to realizing an effective preventive vaccine. We will now publish objective Review articles and more opinionated Perspective pieces from influential scientists and other leaders in the field on topics integral to AIDS vaccine research and associated disciplines.

This won't come at the expense of accessibility; our aim is to appeal to a diverse range of readers, so that there will be something for all audiences, irrespective of their degree of scientific training. We hope that the more socially-inclined articles will help remind scientists why they are doing what they are doing, and that the more molecular biology- and immunology-oriented pieces will challenge non-scientists to increase their knowledge base.

To illustrate, this issue of *IAVI Report* focuses on topics related to the transmission of and early events after HIV infection, and features Review and Perspective articles in those areas. Melissa Pope writes about dendritic cell-dependent events immediately after HIV has breached the mucosal barrier; a better understanding of these events will provide insight into what may be a decisive window of opportunity to stamp out potential infection. Robin Shattock writes about the accelerating momentum in microbicide research, the difficulties that come with a growing field, and the parallels with other HIV/AIDS prevention and treatment initiatives.

We hope you enjoy and are informed by these and the regular articles featured in our re-design, and we welcome any feedback and suggestions at *iavireport@iavi.org*.

## Env immunogens contribute to AIDS vaccine protection in simian models

Following infection with HIV, neutralizing antibodies and cytotoxic T lymphocytes (CTLs) are raised to specific Env epitopes. But global Env diversity poses a significant challenge for the development of an effective AIDS vaccine, and the utility of including an Env immunogen as a vaccine component has been questioned.

In previous studies, vaccine-elicited Env-specific cellular immune responses have protected monkeys challenged with both simian immunodeficiency virus (SIV) and simian-human immunodeficiency virus (SHIV) expressing Envs identical to the immunogen. Norman Letvin and colleagues have now looked to see if an HIV Env immunogen contributes to protective immunity against challenge with a pathogenic SHIV-89.6P having a genetically disparate Env (*J. Virol.* **78**, 7490; 2004).

The vaccination strategy employed a plasmid DNA prime and a recombinant replication-defective adenovirus (rADV) boost. To determine whether an Env immunogen induces protection against a genetically disparate challenge SHIV, six monkeys were vaccinated with one of three different DNA plus rADV strategies: (i) Mock (Gag-Pol-Nef with no Env), (ii) Env-matched (Gag-Pol-Nef with SHIV-89.6P Env), and (iii) Env-mismatched (Gag-Pol-Nef with HXB2/Bal Env). All monkeys were then challenged 12 weeks later with SHIV-89.6P. A control group of six unvaccinated naïve monkeys were also challenged with SHIV-89.6P.

All controls suffered a profound loss in $CD4^+$ T cells, which is the usual outcome of SHIV-89.6P infection in naïve animals. Four of six monkeys vaccinated with Gag-Pol-Nef with no Env were able to mitigate $CD4^+$ T-cell loss after SHIV-89.6P challenge, as expected from previous studies. The two groups of vaccinated monkeys that received Env (matched or mismatched) in addition to Gag-Pol-Nef immunogens putatively demonstrated a significantly better mitigation of $CD4^+$ T-cell loss than the group of monkeys that received only Gag-Pol-Nef immunogens without Env. Viral replication in monkeys after SHIV-89.6P challenge was monitored by quantitating viral RNA load in plasma by using a bDNA assay. Here they found that the unvaccinated control animals had significantly higher peak viral loads than the vaccinated animals. However, there was no significant difference between the three vaccinated groups, whether they received Env immunogen or not.

Env-immunized monkeys developed high-titer antibodies to their cognate Env (SHIV-89.6P Env or SHIV-HXB2/Bal Env), but plasma samples from them failed to neutralize SHIV-89.6P virus *in vitro*, so they were unable to demonstrate that neutralizing antibodies directed against SHIV-89.6P contributed to viral containment after challenge. However, peripheral blood mononuclear cells from monkeys that received either matched or mismatched Env immunogens developed several-fold higher ELISPOT responses to SHIV-89.6P and HXB2/Bal, respectively, than did the monkeys that received the mock Env injections.

The authors conclude that the results suggest a strong association between the generation of postchallenge Env-specific T-cell immunity and the inclusion of either matched or mismatched Env immunogens in the vaccine regimes of the tested monkeys. But given the limits of the small numbers of animals providing comparison in the paper, the only apparent advantage of including Env is the broadening of the vaccine-elicited antiviral cellular immune responses. It is also of interest that the responses are cross-reactive.

## Natural history of incidental HIV infection after canarypox vector AIDS vaccination

Many researchers think that AIDS vaccine candidates that predominantly elicit cellular immune responses may not achieve sterilizing immunity but may modulate disease progression. So far, data on human subjects who became infected incidentally with HIV while participating in AIDS vaccine clinical trials were from trials of recombinant envelope subunit vaccines, not viral vector-based immunogens. Celum and colleagues reported on US National Institute of Allergy and Infectious Diseases (NIAID)-sponsored safety and immunogenicity trials of several canarypox vector (ALVAC) AIDS vaccine candidates constructed with clade B sequences (*J. Infect. Dis.* **190**, 903; 2004). They looked into the natural history of early HIV infection among participants who received an ALVAC vaccine with or without a booster dose of recombinant gp120 or gp160, compared to that among placebo recipients.

A total of 1,497 study participants enrolled in nine Phase I and two Phase II canarypox AIDS vaccine prime-boost trials, of whom 1,257 were vaccinees and 240 were placebo recipients. Overall, 30 participants (2%) became HIV infected after enrollment, 18 of whom consented to enroll in breakthrough-infection protocols.

HIV seroincidence rates among canarypox HIV vaccine and placebo recipients were similar and comparable to those in the HIVNET Vaccine Preparedness Study (1.38 HIV infections/100 person-years). Vaccinees and placebo recipients who became infected with HIV were not different with respect to the proportion with symptomatic seroconversion and rate of disease progression, suggesting that the natural history of early HIV infection among vaccinees is similar to that of placebo recipients and historical cohorts.

The authors caution that reliable estimates of efficacy in reducing HIV infection rates cannot be derived given the small sample size of Phase I and II trials and the differences in immunogens and immunization schedules across protocols. They also point out that a series of large, randomized, controlled studies in diverse populations will be required to define the effect of preexisting HIV immunity on the long-term sequelae of HIV infection.

It is important to note that the authors of this article did not include any data on the vaccine-elicited immune responses in the volunteers, because the data could be potentially misinterpreted. Therefore, while the infection data are quite discouraging with regard to this particular test vaccine, the data do not address the antiviral potential of a vaccine-elicited cellular immune response.

## Specific immune responses in primates vaccinated with adenoviral vector vaccines

Recombinant serotype 5 human adenoviruses (Ad) are promising vectors for delivering HIV antigens. Hildegund Ertl and colleagues (*J. Virol.* **78**, 7392; 2004) report on new chimpanzee E1-deletion Ad vectors (AdC6 and AdC7) constructed to express a truncated form of HIV-1 Gag and developed to circumvent preexisting immunity to Ad 5 in many adults. The vaccines were tested for induction of T-cell responses in mice and nonhuman primates (NHP) in triple immunization protocols that included a heterologous human E1- and E3-deletion Ad 5 vector with the same truncated Gag. The E1-deletion reduces transcription of Ad antigens which results in loss of cell death upon infection and thus sustained antigen presentation, but also in an immune response that is more focused on the transgene product.

In previous studies, simian Ad vectors of serotypes C68 and C6 have induced potent transgene-specific $CD8^+$ T-cell responses in mice and these responses could be boosted using heterologous vectors. Ertl and colleagues expanded these studies to two triple immunization protocols.

In mice, each booster immunization increased the frequencies of $CD8^+$ T cells by two to threefold. The Gag-specific $CD8^+$ T cells were stable, especially after the third immunization. In NHP, Gag-specific T-cell responses were readily detectable after the first booster immunization in all animals, although the frequencies of responses differed between the animals. They assumed this variability was in part due to the short Gag transgene used in this proof-of-principle study, encoding a limited number of T-cell epitopes. The longevity of the T-cell responses in NHP also differed but was remarkably stable over the four month period between the first and the second boosters.

The second booster immunization in NHP transiently increased interferon gamma-producing $CD8^+$ T cells but overall the yield was not as impressive as the results observed in mice. The frequency of IL-2-producing T cells (presumably $CD4^+$ T cells) was significant in most animals after three immunizations. IL-2-secreting cells are viewed as critical to the success of AIDS vaccines.

The authors conclude that, within the limitation of Gag-encoding Ad vaccines for which preclinical challenge models are not available, the results of these studies are promising because frequencies of Gag-specific $CD8^+$ T cells obtained with the triple immunization protocols were markedly higher in the high responder animals than those obtained with previous SIV-HIV protocols.

## Vaccine-induced CTLs contain highly pathogenic immunodeficiency virus infection

The significance of CTLs in the control of immunodeficiency virus infections has been demonstrated by associations between CTL activity and by control of viremia in primary HIV infection in humans, and $CD8^+$ T-cell depletion experiments in SIV-infected macaque models. However, it remains unclear if vaccine-induced CTL responses could control chronic SIV disease progression or, ultimately, HIV replication in humans.

Tetsuro Matano and colleagues (*J. Exp. Med.* **199**, 1709; 2004) now provide evidence that vaccine-induced CTLs can result in the containment of SIVmac239 infection in non-Indian macaques; the vast majority of previous studies have used Indian macaques. The authors say that the challenge virus they use, SIVmac239, is a more realistic challenge virus than SHIV-89.6P because the former induces chronic disease progression (analogous to HIV infection) rather than the acute $CD4^+$ T-cell depletion induced by the latter. Eight rhesus macaques were vaccinated with a DNA prime followed by a single boost with a recombinant Sendai virus vector (SeV) 6 weeks later; both vaccine components expressed SIVmac239 Gag. All macaques were challenged intravenously with SIVmac239 13 weeks after the SeV boost. Four unvaccinated control macaques developed high peak viremia on day 10 after challenge and maintained relatively high plasma viral concentrations, while five out of the 8 vaccinated macaques controlled replication of the highly pathogenic challenge virus; plasma viremia became undetectable after week 5 and peripheral $CD4^+$ T cells were maintained.

At week 2 after challenge, the investigators detected anamnestic Gag-specific $CD8^+$ T-cell responses in all of the vaccinated macaques, indicating efficient secondary responses during the acute phase of infection. They found no neutralizing activities in plasma against SIVmac239 in any of the controls or the vaccinees at weeks 5 or 12 after challenge, suggesting that neutralizing antibodies were not essential for the control of SIV replication observed. The SIV gag region in the viral genomes obtained from plasma RNA at week 5 after challenge was sequenced to determine whether vaccine-induced Gag-specific T-cell responses exerted a selective pressure on the virus. The numbers of amino acid changes per clone in the vaccinated macaques were significantly higher than those in the unvaccinated, which may reflect the immune pressure exerted by vaccine-induced Gag-specific T-cell responses.

All of the macaques that controlled SIVmac239 replication showed consistent amino acid changes in Gag, some of which conferred diminished replication efficiency of the viruses, *in vitro* as well as *in vivo*, compared with the wild-type SIVmac239.

The authors conclude that vaccine-elicited CTLs can "cripple" the virus by imposing a fitness cost, and results in the containment of replication of a neutralization-resistant, highly pathogenic immunodeficiency virus that is not contained in the natural course of chronic infections. The data in this paper are notably different from data that have been previously presented with the SIVmac239 challenge model. It has typically been notoriously difficult to demonstrate that vaccine-elicited cellular immune responses can mitigate infection with this challenge virus. However, the reported observations could likely be influenced by the use of non-Indian macaques in which infection with SIVmac239 is attenuated compared with the usually used Indian macaques. Nonetheless, the data support a positive role for vaccine-elicited cellular immune responses in the mitigation of the SIV challenge infection.

# Vaccine Briefs

## G8 endorses plan to accelerate AIDS vaccine development

US President Bush hosted the 2004 Group of Eight (G8) summit of developed nations at Sea Island, Georgia on 8-10 June. The summit endorsed the establishment of a global AIDS Vaccine Enterprise to accelerate efforts to develop an AIDS vaccine that seeks to further encourage scientists from around the world to create vaccine development centers, with the headquarters in the US. Other goals are to support the standardization of laboratory test systems to facilitate comparison of trials from different countries and to build an integrated clinical trials system. The plan will also seek to eliminate red tape so that regulatory agencies from different countries can more easily recognize clinical trials and data. The Enterprise will also look to stimulate dedicated vaccine manufacturing capacity.

An international group of scientists published a "Policy Forum" in *Science* magazine last year (*Science* **300**:2036, 2003) calling for a virtual consortium to accelerate AIDS vaccine development by enhancing coordination, information sharing, and collaboration globally. The G8 has now endorsed this concept in a statement calling on the Enterprise to "establish a strategic plan that would prioritize the scientific challenges to be addressed, coordinate research and product development efforts, and encourage greater use of information sharing networks and technologies. This plan should serve as a blueprint for helping to align better existing resources and to channel more efficiently to the needs at hand new resources as they become available." For more details, visit the G8 Web site at *www.g8.gc.ca/*.

## GenVec and NIH move AIDS vaccine candidate into clinical trials

GenVec, Inc., a biotech company based in Gaithersburg, Maryland, announced last month that the Vaccine Research Center of the National Institute of Allergy and Infectious Diseases (NIAID) has initiated a Phase I clinical study to test an AIDS vaccine candidate that uses GenVec's proprietary modified adenovirus particles as vectors, consisting of a second generation type 5 adenovirus with E1, E3 and E4 deletions. The vector is currently being used in other unrelated human trials.

The recombinant products used in this trial are composed of four adenoviral vectors (in a 3:1:1:1 ratio) that encode an HIV-1 Gag/Pol fusion polyprotein from clade B and HIV-1 Env glycoproteins from clades A, B, and C, respectively. The vaccine will be given to 36 healthy volunteers, most from the Washington DC area to determine the "safety, tolerability, immune response of a multiclade HIV adenoviral vector vaccine in uninfected adults." The study will be sponsored, managed and funded by NIAID.

The US$30 million NIH contract covers production of vaccines for both AIDS and SARS, or severe acute respiratory syndrome. This Phase I, dose-escalating, double-blind, placebo-controlled study is designed to assess safety and immunogenicity of a vaccine candidate targeting clades A, B, and C, and intended to induce both humoral and cell-mediated immunity. NIAID Director Anthony Fauci told *The Wall Street Journal* that, while the vaccine candidate did not protect monkeys from infection, they did show a less severe course of disease. For more details, visit the IAVI Database of AIDS Vaccines in Human Trials at *www.iavireport.org/trialsdb/*.

## US Army begins small Phase I trial

AVANT Immunotherapeutics, Inc. announced in May that the Walter Reed Army Institute of Research (WRAIR) has initiated a Phase I clinical trial to assess the safety and immunogenicity of an AIDS vaccine based on AVANT's Therapore technology. Therapore utilizes bacterial toxin proteins to deliver target antigens into human cells to induce cell-mediated immune responses. The WRAIR AIDS vaccine, designated LFn-p24, is comprised of a *Bacillus anthracis*-derived polypeptide called lethal factor from which the toxin domain has been removed (LFn) fused to the HIV Gag p24 protein. The vaccine is aimed at inducing strong and persistent HIV Gag-specific $CD8^+$ T-cell responses.

The placebo-controlled trial is evaluating the vaccine at three escalating dose levels in 18 healthy adult volunteers. Volunteers in each of the three dose groups in the study will receive three intramuscular vaccine immunizations or placebo injections at weeks 0, 4 and 16 and will be followed for at least 36 weeks following their final dose. The trial, under the direction of principal investigator CDR Shirley Lee-Lecher, is being conducted at the WRAIR Vaccine Clinical Research Center in Rockville, Maryland in conjunction with the Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID). WRAIR and NIAID are working together through an established interagency agreement.

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

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## Ad5 Phase IIb Efficacy Trial Announced at the AIDS Vaccine Research Working Group

The 27-28 May meeting of the National Institutes of Health's AIDS Vaccine Research Working Group (AVRWG) saw the first public discussion of plans for a Phase IIB efficacy trial of Merck & Co.'s adenovirus-based AIDS vaccine candidate. Details of the study, a collaborative effort between Merck and the HIV Vaccine Trials Network (HVTN), were presented by Robin Isaacs, director of HIV vaccine clinical trials at the Merck Research Laboratories.

The primary goal of the trial (scheduled to start in the fourth quarter of 2004) is to evaluate whether vaccine-induced HIV-specific $CD4^+$ and $CD8^+$ T cell responses can either provide protection against HIV infection or reduce postinfection viral load in vaccinees who become infected. Isaacs stressed that the trial is not designed to lead to licensure of Merck's vaccine; an additional Phase III efficacy trial will be required if the company decides to seek FDA approval. Given that the vast majority of current AIDS vaccine candidates seek to induce HIV-specific T cell responses, this trial also promises to provide information crucial to advancing the field as a whole.

The trial plans to enroll 1,500 homosexual men and heterosexual women with a high risk of sexual exposure to HIV infection (individuals whose primary risk factor is injection drug use are excluded). Isaacs noted that Merck specifically aims to enroll at least 350-450 women. Recruitment will take place at HVTN study sites located in North America, South America and the Caribbean. Immunizations are at weeks 0, 4 and 26; the vaccine is Merck's adenovirus serotype 5 (Ad5) vector, most likely containing HIV *gag*, *pol* and *nef* (at the time of the AVRWG meeting a final decision on which immunogens to include had not been made). One problem with the Ad5 construct is that many people have been naturally exposed to adenovirus (which causes severe colds) and therefore have high titers of anti-Ad5 neutralizing antibodies. Because the purpose of the trial is to optimize the conditions for inducing HIV-specific T cell responses, individuals with baseline neutralizing antibody titers over 1:200 will be excluded. Isaacs reviewed Phase I and II data showing that the Ad5 vaccine induced HIV-specific T cell responses in 63-75% of individuals with antibody titers below the 1:200 cut-off, which represents the highest response rate yet reported for any T cell-based vaccine.

In terms of endpoints, a total of 50 HIV infections are anticipated over the 3.5 year duration of the study. Isaacs reported that 30 infections would provide 80% power to detect a greater than 1 log difference in post-infection viral load set-point between vaccine and placebo recipients, thereby allowing an early interim look at vaccine efficacy. The final total of 50 infections provides 80% power to detect 60% efficacy in preventing persistent HIV infection. These assumptions are based on a predicted HIV incidence of 2.5% per year among high-risk homosexual men and 1.25–1.5% among high-risk heterosexual women. The study design allows for a drop-out rate of 10% during the first year and 5% per year thereafter.

During the discussion period, members of the AVRWG expressed strong support for the trial. There were some questions from Jerry Sadoff (Aeras Global TB Vaccine Foundation) regarding whether the study was statistically powered to achieve its goals but the consensus appeared to be that the design would hold up unless the assumptions regarding HIV incidence and drop-out rates prove seriously erroneous.

## Improvements to the Thai Prime-Boost Trial Recommended

At the same meeting the AVRWG also discussed a series of recommendations to improve another efficacy trial, the Phase III evaluation of a prime-boost protocol involving Aventis-Pasteur's ALVAC vCP1521 canarypox vector and VaxGen's subtype B/E AIDSVAX vaccine, currently ongoing in Thailand under the aegis of the US Military HIV Research Program. There has been controversy regarding this trial since a series of exchanges between scientists were published in the journal *Science*. At the previous AVRWG meeting in January 2004 it was recommended that a subcommitte–chaired by Scott Hammer (Columbia Presbyterian Medical Center) and comprising Larry Corey (HVTN), Jerry Sadoff and statistics adviser Steve Self (Scharp Statistical Center, HVTN)—review the study design and suggest improvements. At the May meeting, Hammer presented the four key recommendations of this group:

- The trial's secondary endpoint of reduction in postinfection viral load set-point should be made a co-primary endpoint with protection against infection. Fifty viral load endpoints would provide 90% power to detect a ≥1 log difference in viral load and 80% power to detect protective efficacy of 60% (com pared to 90% power to detect protective efficacy of 50% in the current design). This would result in at least a 50% reduction in sample size (from 16,000 to 8,000 or less).
- The method of assessing the viral load endpoint should be better defined (e.g. the geometric mean of 2-3 HIV RNA values post-infection to define early set-point).
- Immunogenicity (especially T-cell response) data from 200-300 vaccinees and 100 controls should be collected and provided to the Data Safety Monitoring Board (DSMB) in real time during the trial. However, such data should not be part of any guideline for prematurely stopping the study.
- A futility analysis should be framed to give the DSMB criteria for terminating the trial early if the goals cannot be met (e.g., due to slower recruitment, lower HIV incidence, or higher lost-to-follow-up rate than predicted).

At the following AVRWG meeting, held in Lausanne on Sept 2, Jorge Flores from NIAID presented the response of the RV144 investigator team to these recommendations. The team agreed to elevate viral load to a co-primary endpoint, but declined to consider a reduction in sample size in order to maintain the original statistical power to detect a 50% reduction in acquisition of infection and "as a safeguard for a decrease in infection rate." There will be extended follow-up of volunteers that become infected and a composite endpoint including clinical events, time to initiation of ART, CD4 counts and viral load is being developed. Immunogenicity data will be collected in a separate cohort of volunteers "to avoid perturbation of the ongoing trial." A plan for defining operational futility (i.e. circumstances under which the trial would be stopped if it cannot reach its goals) is being developed. These proposed changes are now being discussed with the Thai National Vaccine Committee and local Institutional Review Boards.