IAVI Report

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SPECIAL REPORT:
Women and AIDS Vaccines

This issue of the *IAVI Report* is devoted to women and gender-related issues in AIDS vaccine research. It's a focus that could raise eyebrows: What is there to talk about? After all, vaccine science has rarely paused to consider gender differences, and has rarely had to. Successful vaccines for polio, tetanus and many other infectious diseases were made without considering the ebb and flow of sex hormones or the male and female genital mucosae.

But unlike any of these diseases, HIV is a sexually transmitted virus. And while HIV disease progresses in similar ways in men and women, there are also some differences in how it interacts with men's and women's bodies.

In the pages that follow, we report on some of the issues that arise when looking beyond a gender-neutral view of HIV. We begin with two articles focused on HIV in women (one on mother-to-child transmission [MTCT], the other on HIV immunity) and then move to an overview of data on gender differences that may be relevant to AIDS vaccine development. Interspersed among these feature articles are interviews with researchers at the front lines of AIDS vaccine trials, who give testimony to both the extreme vulnerability of young women—the fastest growing risk group in many parts of the world—and to the implications of their high risk and lower societal status for vaccine trials.

On the scientific front, what emerges first are questions, not answers. As with other diseases, data

continued on 2 ▶

A LETTER FROM IAVI: NEW YORK OFFICE REOPENS AFTER WORLD TRADE CENTER ATTACK

All of us at IAVI are deeply saddened by the tragic events of 11 September. Our hearts go out to those who have lost loved ones.

As many of you know, IAVI's New York office is located only a few blocks from the World Trade Center, although far enough away to have been spared damage. We are fortunate to report that IAVI's staff and the visiting researchers attending a scientific advisory committee meeting at the time are all safe.

We extend our deepest thanks to partners, colleagues and friends around the world for their warm outpouring of concern and reassurances. Although headquartered in New York, IAVI is truly an international organization. Now more than ever we will need the support of our staff, consultants, partner organizations, colleagues and friends around the world, and we look forward to continuing to work with them towards the goal of finding a vaccine against AIDS.

Following the attack, lower Manhattan was closed for the week. We apologize for any delays in responding to messages during this time. While we were able to conduct limited operations in space generously provided by the Rockefeller Foundation, our electricity, phones and computer server were down. On 20 September, we reopened the New York office, after engineers certified that the building was structurally sound and services to the area had been restored.

The events of 11 September interrupted, but by no means halted, our global work. Our science programs continue in Kenya, Uganda, South Africa and India, and our international network continues to build worldwide support for AIDS vaccine research and development.

These events have touched all of us very personally. Moving forward will not be easy, but it is essential. We especially regret that New York staff who had planned to attend the International Congress on AIDS in Asia and the Pacific (ICAAP) in Melbourne, where many of you will be reading this newsletter, are unable to attend, although other members of the IAVI international team will be present.

IAVI's mission—ensuring the development of safe, effective and accessible AIDS vaccines for the world—is one that affirms the value of every human life. The events of 11 September have reinforced for us the importance and urgency of this work. ◆

Inside:

◀ **SPECIAL REPORT** *continued from 1*

> *"Will viral load levels for health and transmission turn out to be gender-specific?"*

on HIV are usually not analyzed by gender unless that is a primary focus of the study. The result: a dearth of direct, comparative information. Most of what is known about gender-specific disease effects comes from natural history cohorts or retrospective analysis of studies undertaken with a different purpose. In "Gender, HIV Transmission and Vaccines," Anne-christine d'Adesky combs through studies that bear on the issue of HIV infectiousness in men versus women. What she finds are compelling arguments for looking at possible gender-specific effects as a matter of course, not a matter of special interest.

What do these arguments look like? There is the well-documented phenomenon that women have lower viral loads than men from the time of acute infection onwards. Researchers are also finding that both men and women have distinct viral populations and immune responses in their genital tracts (and possibly breast milk in women) compared with blood.

These data may seem like a tangle of disparate findings. But there are key lines of inquiry that emerge, many of which span research on vaccines, microbicides, antiretrovirals and MTCT. On the short-list: How do viral load and immune responses vary in the male and female genital tracts, plasma, breast milk, and semen? What defines infectiousness in these different compartments and fluids, each with its own set of immune players?

HIV vaccinologists cannot address all of these basic science questions. But forward-thinking collaborations with other fields can help fill in the picture. One area where this is starting to happen is in research on vaccines to reduce HIV transmission via breast milk. As Emily Bass reports on page 3, some seasoned clinicians are preparing to test whether HIV vaccines given to newborns can help safeguard breastfeeding by HIV-positive mothers, a widespread practice in the developing world both for cultural reasons and because of the health risks associated with formula feeding. Along the way, the researchers are conducting and supporting studies of breast milk immunology and viral load dynamics in trials of other MTCT preventions—knowledge that could help guide evaluation of vaccines in this context.

Turning to AIDS vaccines in adults, the question is whether, and how, gender might impact vaccine efficacy. The working model behind most current strategies (although it is not formally validated) is that without sterilizing immunity, vaccines which control viral load will provide significant benefits in terms of prolonging life, and presumably in reducing transmission. To assess vaccines by these criteria means understanding how much lowered viral load levels will slow disease progression and decrease infectivity. Yet, as d'Adesky describes, a new study of serodiscordant couples in Zambia suggests there are gender differences in how viral load relates to transmissibility. It's a confounding finding—and one which demands closer attention. Will viral load levels for health and for transmission turn out to be gender-specific? Given the existing data, we cannot afford to assume otherwise.

Moving from transmission to acquisition—the step vaccines target—there is now good evidence that immune protection to HIV *can* be achieved. As Richard Jefferys reports on page 9, some of it comes from studies of women in the Kenyan sex worker cohort, who have been providing samples and sharing information about their lives for years. A small minority of them do not seroconvert despite repeated exposure to HIV from many different partners. Since the mid-1990's, researchers have been working to identify both the immune players behind their apparent resistance and the precise regions of HIV that stimulate the protective responses. With their successes will come valuable information for designing and evaluating vaccines.

But when all is said and done, could a vaccine really show significant differences in men versus women? As far-fetched as that may sound, there could now be a first example: an experimental vaccine against HSV-2, the virus that causes genital herpes. As Patricia Kahn reports on page 15, two Phase III trials of GlaxoSmithKline's Simplirix® vaccine suggest that it provides some protection in women, but none in men. While further testing is planned to confirm this finding—and begin looking for explanations—there's a message to heed: vaccines may not always be gender-blind.

Practically speaking, the way to find out is by enrolling enough men and women to power trials for detecting gender differences. But including high-risk women can be difficult in practice. VaxGen's two Phase III trials, the only efficacy studies of an HIV vaccine so far, focus on gay men and IV drug users, resulting in cohorts that are over 90% male. Uganda's Phase I ALVAC trial drew from the military and surrounding communities, resulting in a majority of male participants.

In articles from three disparate settings—two in sub-Saharan Africa and one in New York City—several clinical researchers discuss women's HIV risks and their involvement in vaccine trials. All three share some common themes, starting with the huge impact of women's lower status, poverty and economic disenfranchisement on their vulnerability to HIV. Another is the need for vaccine trial staff to engage in the social context—a practice which has been the exception rather than the rule in research—if they are to successfully recruit and retain high-risk women. It's an approach that stems from the understanding that "high risk" is a catch-all phrase encompassing many factors, including poverty, drug-use, physical and sexual abuse and lack of autonomy.

To get the gender-specific data that are needed, then, vaccine trials will need to address gender both from a scientific and social perspective. This will not move the field away from its scientific goals, but should take us closer to the heart of the matter. ◆

AIDS VACCINES AND HIV TRANSMISSION VIA BREASTFEEDING

BY EMILY BASS

In 1997, the antenatal clinic at Mulago Hospital in Kampala was the site of a clinical trial that launched a thousand hopes for the battle against AIDS in children. HIVNET 012 showed that a simple, cheap regimen of the antiretroviral drug nevirapine (NVP)—one dose to the mother in labor, one to the infant within 72 hours of birth—reduced rates of HIV transmission at delivery by nearly 50%. In sub-Saharan Africa, that could potentially translate into preventing as many as 300,000 infections per year. But this remains a distant goal: even with a drug donation offer from NVP's manufacturer (Boehringer Ingelheim), the treatment has been very slow to move beyond clinicial research settings into local health systems.

And even after birth, most of these children are not out of the woods. Within a year, 10-15% of them will acquire HIV via breastfeeding, whittling away at the successes of early interventions like NVP or AZT (the established but more expensive anti-retroviral that blocks mother-to-child transmission [MTCT]). "700,000 babies get infected in the world each year, up to half through breastfeeding," says Laura Guay of Johns Hopkins University (Baltimore) and Makerere University (Kampala), a pediatrician who has spent more than a decade in Uganda. "Right now, we are desperately in need of something."

The need is so great because, in Uganda and much of the developing world, breastfeeding is still practiced by most HIV-positive women. That's partly due to social stigma: formula feeding can be tantamount to a public declaration of HIV infection. But many women cannot afford formula, or they lack access to clean water or fuel. There are also compelling health reasons, since formula feeding places infants at a higher risk for life-threatening childhood afflictions such as diarrheal diseases, dehydration and malnutrition. Under these circumstances, safeguarding breastfeeding could save more lives than trying to follow the lead of industrialized countries and switch to formula feeding.

That's why Guay and her colleagues at Makerere University are part of the small group of researchers pursuing HIV vaccines as a potential strategy for protecting breastfeeding infants against infection. At press time, Guay and Francis Mmiro, an elder statesman of AIDS research in Uganda, were preparing to submit a trial protocol to both the Johns Hopkins and Ugandan Institutional Review Boards (IRB's)—a first step in the approval process for a Phase I vaccine trial of Aventis Pasteur's canarypox-based vaccine, ALVAC vCP1452, in newborns. If approved, it will be the first neonatal HIV vaccine trial outside North America.

High Hopes and a Lowered Bar

The notion of a neonatal HIV vaccine may sound like a long shot, since there is still no effective adult vaccine. But the bar for protection in infants may be lower: rather than long-term immunity, a neonatal vaccine need only protect for as long as babies are breastfed. While that can last up to two years, work by leading MTCT researcher Ruth Nduati (University of Nairobi), and a large study in Malawi suggest that the greatest risk of transmission is in the first six months. So even a less effective vaccine might have a major impact. Another ground for optimism: while about 15% of breastfed babies become infected, that leaves 85% who do not—an intriguing, apparently innate protection that is still poorly understood (see article, page 4).

The first clinical studies of HIV vaccines in neonates date back to 1993, when a trial led by William Borkowsky (New York University Medical School) tested two different gp120 subunit vaccines and found them to be safe and immunogenic. A few years later, infectious diseases researcher Jack Lambert (then at Johns Hopkins University) launched an NIH-funded neonatal trial of ALVAC vCP205 through the Pediatric AIDS Clinical Trials Group (PACTG 326). This vaccine, based on a canarypox viral vector, was known from Phase I testing in adults to induce cellular immune responses in up to half of all vaccinees. It was also safe in adults, which was key to its selection despite somewhat weak immunogenicity. (Both vCP205 and the related vCP1452 are likely to enter Phase III efficacy trials in adults within the next 1-2 years)

The PACTG 326 trial, conducted at sites throughout the U.S., followed 27 mother-infant pairs, all of whom received antiretroviral treatment to prevent HIV transmission before and during birth; babies were delivered vaginally or by caesarian section depending on clinical indications. Newborns were immunized with vCP205 within 72 hours of birth and then again at 4, 8 and 12 weeks. While up to 50% of the babies showed cellular immune responses to the vaccine (in proliferation and CTL assays), says Lambert, the responses often hovered near the lower threshold of detection. All babies were exclusively formula-fed and none became infected during the trial.

Aiming to improve on these results, Lambert (now at the Institute of Human Virology, University of Maryland) is overseeing the next phase of PACTG

continued on 5 ▶

IS THERE A ROLE FOR VACCINES IN PROTECTING INFANTS AGAINST HIV IN BREAST MILK?

Holding HIV At Bay: What Keeps Exposed Babies Uninfected?

One of the biggest puzzles in understanding mother-to-child transmission of HIV is why the majority of babies born to HIV-infected women remain uninfected *in utero*, at birth and—perhaps most remarkably—during breastfeeding. It's even more remarkable in view of studies suggesting that cell-free viral load in breast milk can vary from undetectable to more than 200,000 copies per ml, meaning that a breastfeeding infant may ingest up to millions of viral copies each day (see *J Infect Dis.1998;*177:34).

This apparent resistance puts infants in the compelling category of exposed, seronegative (ESN) individuals (see article, page 9) who can repel or effectively control HIV despite repeated exposures. Katharine Lazuriaga and Sarah Rowland-Jones have both documented cases of infants apparently clearing a transient HIV infection. It is these immune defenses which vaccine researchers seek to boost, or mimic, with a neonatal vaccine. But there is little hard data on just what they are and how this apparent protection works.

New research by Marta Marthas (California Regional Primate Research Center, University of California, Davis) could help fill in the picture. Marthas showed earlier that subcutaneously administered SIV hyper-immune serum protects newborn monkeys against infection by orally delivered SIV-mac 251 (*J. Infect. Dis.*1998;177:1247). This spring, she returned to the issue with a multiple low-dose challenge study designed to approach conditions of breast milk transmission—the first primate study to tackle this problem.

Working with 40 neonatal macaques, Marthas is using oral challenges in groups of animals. The challenges, given three times daily, five days a week, are 10-100 fold lower than the standard single oral challenge. So far, she says, the majority of the animals are getting infected, including all four given the highest dose and one out of four (so far) in the lowest-dose group. The uninfected animals wil be sacrificed after three months and autopsied to look for signs of latent SIV infection (since cultures from a few animals yielded virus after 8-12 weeks) and SIV-specific immune responses in the tissues. Similar studies done on adult female macaques who remained seronegative after a single, low-dose vaginal challenge showed signs of SIV infection and SIV-specific proliferative responses upon autopsy two years later (*J. Virol.*1998;72:10029).

In one sub-study, Marthas will evaluate immune responses and protection against low-dose challenge in infant rhesus monkeys vaccinated with an SIV-MVA (made by Pat Earle and Bernie Moss at the National Institute of Allergy and Infectious Diseases) or an SIV-ALVAC construct (a simian version of ALVAC vCP205 containing *env*, *gag* and *pol* from SIV-MAC 239, made by Aventis Pasteur). She is keen to find out whether the responses to these vaccines will be different with a repeated low-dose compared with the standard single, high dose challenge. "If you give multiple challenges after vaccination and get transient or abortive replication, can you boost the immune responses that were induced by the vaccine? Perhaps," says Marthas. Based on what she's seen so far, including late viremia or compete protection of some vaccinated infants, Marthas thinks this model could be a better way to test vaccines. "It's made me think we might be throwing out some vaccine candidates prematurely based on results of high-dose challenge studies."

In human studies, cord blood samples provide a valuable window into immune defenses that develop *in utero*, when the fetus is exposed to viral particles and proteins that cross the placenta. It's here that Louise Kuhn (Columbia University) and her collaborators Anna Coutsoudis (University of Natal), Glenda Gray (Chris Hani Baragwanath Hospital, Johannesburg) and Mario Clerici (University of Milan) found striking evidence that T-helper cell responses correlate with protection against MTCT.

Kuhn collected cord blood samples from 86 infants in a vitamin A supplementation trial in South Africa. Of the 86, 33 (38%) had HIV-specific CD4+ T-helper responses at birth. Three of these responding infants were born HIV-positive, 28 were negative, and 2 were lost to follow-up. Significantly, none of the infants with T-helper cell responses at birth became infected during breastfeeding. In contrast, 6 out of 53 infants (11%) lacking CD4+ responses were infected prior to delivery and 17% were infected during labor or at delivery.

In a provocative follow-up study, Kuhn looked for HIV-specific CD4+ T-helper cells in infants born to mothers who received AZT/3TC before and after delivery. Surprisingly, none of the infants in this study showed these responses, an outcome that did not correlate with maternal viral load. Kuhn speculates that this may be due to some other interaction between the antiretrovirals and co-stimulatory factors, such as cytokines or antigen-presenting cells, needed for an anti-HIV immune response. The possibility that short-course antiretrovirals for the mother could influence the infant's ability to mount immune responses is yet another argument for interventions to protect infants during breastfeeding. Kuhn and others emphasize that the helper responses reappear in infants, and that these findings do not imply that ARVs should be withheld from HIV-positive pregnant women.

As for why some infants develop these T-cell responses and others do not is, Kuhn calls this "the million dollar question." Their presence does not appear to correlate with maternal viral load, CD4+ T-cell count or gestational age at delivery. "It might have something to do with the way the virus is presented *in utero*, with the presence of specific epitopes, or with some kind of interaction between the mother and the child genetically," says Kuhn, adding that "it's all just speculation." She is now following up with a closer look at neonatal correlates of protection, and with a study of nevirapine and its effects on T-helper immune responses.

— E.B.

326, which uses a newer canarypox-based vaccine (ALVAC vCP1452) in a prime-boost regimen with VaxGen's gp120. To date, over 50% of a planned 24 children have been enrolled at PACTG sites throughout the country. Lambert says that the immune responses so far do not look any stronger than with vCP205, but complete data is not yet available.

HIVNET 027, the proposed NIH-funded Phase I trial at Mulago Hospital, will use ALVAC vCP1452 in 50 mother-infant pairs (40 vaccine and 10 placebo). All women and infants in the trial will receive the short-course NVP regimen rather than AZT. Another key difference to the North American studies: if experience holds up, most of the women in the Ugandan trial will choose to breastfeed, so the babies will be exposed to HIV after vaccination. Whether (and how) this affects their immune responses is a question Guay and Mmiro, the trial's two principal investigators, hope to tackle in the study, along with monitoring safety and immunogenicity. They will also monitor the responses to standard childhood vaccines, to be given at staggered intervals between the experimental vaccinations (to help pinpoint the source of any side effects).

Arriving at this plan has been a process extending over several years. The researchers knew that acceptance of a neonatal trial in Uganda would require a vaccine already well-tested for safety in adults and babies, which meant waiting until PACTG 326 was near completion. They also debated whether to go with the existing vCP1452 (based on HIV subtype B) or—since subtype A predominates in Uganda—to wait for the new subtype A vCP1452 developed by Aventis Pasteur. In the end they opted for the former, since it will take time for the subtype A vaccine to accrue a comparable safety record in adults. (The Walter Reed Army Institute for Research [WRAIR] is expected to submit a Phase I trial protocol of this vaccine for approval in Uganda within the next few months.) Another factor in the decision was the finding that some Ugandan adults showed cross-reactive immune responses in a recent trial of vCP205.

HIVNET 027 will also measure CD8+ T-cell responses, using a modified CTL assay which focuses on a restricted number of antigens. That's due to a key limitation of working with neonates: since blood samples are limited to 2-5ml, it's rarely feasible to repeat a CTL assay or confirm it with other tests. "It's sort of a one-shot deal," admits Guay. They may turn to ELISpot assays in the future, she says, but for now the assay is not standardized well enough for use on neonates.

Infants who become HIV-infected during the course of the trial will continue to be monitored for viral load and immune responses, with other newborns enrolled to maintain a constant sample size. They will also continue to receive vaccine after testing positive, another key difference from the US trial. "We want to know about safety in kids who are already infected," explains Guay, since a neonatal vaccine would presumably be used in settings where HIV infections cannot be definitively diagnosed at birth (which requires PCR-based testing). The infected infants will be offered PCP prophylaxis, free medication for any illnesses and nutritional support, and will be referred to outside facilities, none of which offer ARV treatment at this time. The same care will be available to all mothers. For now, researchers say that antiretroviral treatment is not sustainable. "The research program has a short life of one to three years," says Francis Mmiro. "If we start these children now on antiretrovirals, who is going to take over?"

Now it's all up to the committees on the approvals pathway. Once the US and Ugandan IRB's have signed off, the protocol goes to the Ugandan vaccine review committees for science and ethics and to the National Council on Science and Technology. The final step is approval by the office of the President. Although this process took two years for the first canarypox trial, Guay and Mmiro hope for greater speed this time around in light of the country's prior experience and the increasing amount of HIV vaccine work in Uganda.

A Need for New Solutions

Studies like HIVNET 027 are part of a growing movement to address breastfeeding transmission. It's a movement fueled by the acknowledgement that formula feeding is not an option in many parts of the world, and that the benefits of breastfeeding may outweigh the risk of HIV transmission. In one study by Ruth Nduati, formula- and breastfed infants had comparable mortality rates—although from different causes—after two years.

Current WHO guidelines for HIV-infected mothers suggest exclusive replacement feeding when it is "acceptable, feasible, sustainable and safe. Otherwise, exclusive breastfeeding is recommended during the first months of life." The Ugandan National MTCT Plan calls for three months of exclusive breastfeeding, followed by a switch to formula. But on the ground, each woman makes her own decision about what is feasible. At Upper Mulago, among the 35% of women who initially opt to formula feed, 56% do not come back to the clinic at six weeks to re-stock their formula supply.

To help get a better picture of what happens during breastfeeding transmission, WHO released a draft in June 2001 of its first guidelines for studies on breastfeeding and HIV transmission. The document notes a serious lack of information and observes that "nearly all studies of transmission through breast-

continued on 6 ▶

◀**HIV VACCINES AND BREAST MILK TRANSMISSION** *continued from 5*

feeding have used customary but now inadequate methodologies." It points out, for example, that studies frequently don't distinguish between exclusive versus mixed breastfeeding. That's an important distinction, since exclusive breastfeeding—which means that infants receive no other fluids for their first 12 months—may carry a lower transmission risk than mixed feeding. The guidelines also call for collection of complete data on breast health.

Studies by the teams of Ruth Nduati and Anna Coutsoudis (University of Natal) have shed light on several important issues, including the relationship between cell-free viral load in breast milk and transmission risk, and on how transmission risk relates to volume of breast milk ingested. But there are still many open questions—including the exact relationship between viral load in breast milk versus plasma, and whether the latter is a useful correlate of infectiousness in a fluid that teems with immune defenses.

Several research groups are now focusing on these questions. For example, scientists at the Uganda Virus Research Institute in Entebbe are launching a study examining the relationship between mastitis and viral load in breast milk, and asking whether breastfeeding from the non-inflamed breast reduces transmission risk—the type of simple solution that can give additional prevention at no cost.

In the meantime, a flurry of trials are testing new interventions for the infants, with antiretroviral prophylaxis topping the list. For example, this year's multicenter SIMBA trial, which includes a site in Uganda, will test whether weekly doses of NVP or 3TC given for six months can reduce breastfeeding transmission.

A few researchers are also looking at immune-based therapies. One strategy being pursued by Brooks Jackson (Johns Hopkins) and including Guay and Mmiro, is to test whether treatment of newborns with a cocktail of antibodies against HIV (HIV immune globulin, or HIVIGLOB) can provide some protection in the weeks or months after birth, when the risk of transmission seems to be highest. This might mimic the antibody protection that appears to be at work with the Hepatitis B vaccine, which protects 90% of infants who receive it at birth.

Following on early studies that showed HIVIGLOB to be safe and well-tolerated in both pregnant women and newborns, an upcoming NIH-sponsored Phase III study in Uganda (co-sponsored by the Ministry of Health) will compare HIVIGLOB given to pregnant women at 37-38 weeks, along with a single dose to babies within 18 hours of birth, with two different NVP regimens. The protocol is in the final stages of the approval process, and is expected to start enrolling in October. Here, too, a CDC-sponsored substudy of this trial will pose a host of basic science questions about breast milk immunology.

An Expanding Field

For now, the US and proposed Ugandan ALVAC trials are the world's only neonatal vaccine studies—but there's a push to build interest. This October, the Elizabeth Glaser Pediatric AIDS Foundation will sponsor a two-day meeting on the science and immunology of pediatric vaccines in Dedham, Massachusetts. "A lot of people are thinking about vaccine trials, but very few are thinking about pediatric trials," says Jeff Safrit, the senior program officer at EGPAF who is planning the conference, which will bring together 20-25 mostly US-based researchers, for a mini think-tank. Safrit is excited about developments like polio and measles vectors. "The potential to use these in kids is just amazing."

These trials could bring insights to the adult field, too. In theory, pediatric vaccine efficacy trials could be much more straightforward that adult trials, due to the high incidence of

MOTHER-TO-CHILD TRANSMISSION RATES OF HIV*

Study	Group	Rate of infant HIV infection (%) at:					
		Birth	1.5 mo.	3 mo.	6 mo.	15-18 mo.	24 months
SOUTH AFRICA[1]	*Breastfed* (n = 394)	6.9	19.9	21.8	24.2	31.6	---
	Formula (n = 157)	7.6	18.0	18.7	19.4	19.4	---
KENYA[2]	*Breastfed* (n = 191)	7.0	19.9	24.5	28	---	36.7
	Formula (n = 193)	3.1	9.7	13.2	15.9	---	20.5
BRAZIL[3]	*Breastfed* (n = 168)	---	---	---	---	21	---
	Formula (n = 264)	---	---	---	---	13	---

** Infants were either breastfed (predominantly mixed breastfed) or fed formula (never breastfed).*

1. Coutsoudis et al., *AIDS. 2001;*15:379
2. Nduati et al., *JAMA*. 2000;283:1167
3. Tess et al., *AIDS. 1998;*19:189

Note: This table only includes cohorts that had at least 100 infants in each of the two feeding groups.
Table from: H.M. Coovadia and A. Coutsoudis, *AIDScience* 1 (http://www.aidscience.com/Articles/aidscience004.asp). Reprinted with permission.

continued on 14 ▶

Women and HIV in Kenya

AN INTERVIEW WITH

Dorothy Mbori-Ngacha

Dorothy Mbori-Ngacha is a pediatrician and senior lecturer at the University of Nairobi, with training in epidemiology from the University of Washington. She has been involved in mother-to-child transmission and perinatal trials for most her research career. Mbori-Ngacha is also senior clinical advisor to the Kenyan AIDS Vaccine Initiative in Nairobi, which is now conducting Phase I studies HIV vaccine studies through an IAVI-sponsored collaboration between the U.K.'s Medical Research Council, Oxford University and the University of Nairobi.

Can you tell us about the epidemic in Kenya and what puts women at such high risk?

In Kenya the HIV prevalence in men and women is similar. But in young people between 15 and 24, there is a big gap—many more women are infected. In towns with high prevalence the risk to young women is 3 times higher than for young men. Since the epidemic in women starts earlier, they are dying very young.

This has to do with the status of women. Because of poverty, young girls have older partners, who are more likely to be infected. Early in the epidemic many men felt that their risk was lower with sexually naive partners. So older men targeted younger women.

Young women who are poor, who are less educated, don't have a voice. A poor family with a boy and girl may push out the girl and encourage the boy to go on with school. As we say in our part of the world, women don't belong to the family. A woman will get married and go off to another family.

In our work, we see that women tend to be at risk not because of their own behaviors, but the behaviors of their partners. You don't have to have multiple partners—one is enough, particularly in high prevalence areas. Many women know that their partners have other partners. But he is your provider, so it is very difficult. Our society says, oh, men are like that. Just accept it. What's the big deal? But now, women can stay in one relationship and still get AIDS.

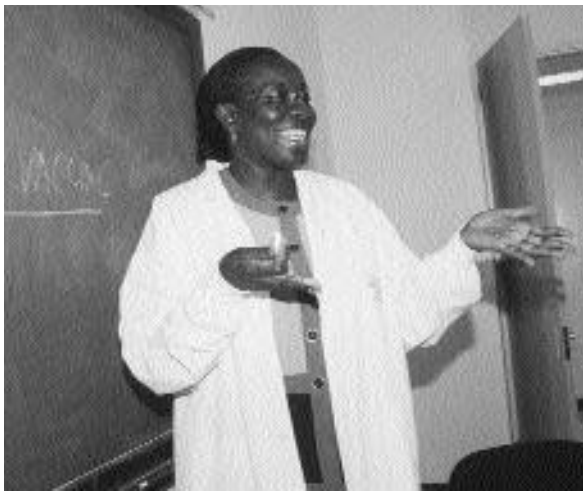
Can you tell us about enrolling women for the HIV vaccine trial?

We felt very strongly that we wanted to include women because they are at such high risk. If we have a vaccine that hasn't been tried in women, how are we going to translate that for women in a timely way? We lobbied hard at our national regulatory body.

But we didn't get many women volunteers—only 2 out of 18 participants. We have our volunteer who went public, Dr. Pamela Mandela. But she's not a typical woman. She's well-educated and has a lot of confidence in herself, which your typical Kenyan woman does not. Many women don't have that capacity to make the decision themselves. They said, I might be interested but I need to discuss this with my partner, or with some experts. For the men, once they've decided, it's done. They may choose to inform their wives or partners, but the decision is theirs.

Another thing that came up for women is the fertility issue. Women need to be clear that they will not have a baby in the next year and a half. That makes many of them think twice.

Will these issues make it hard to enroll women in Phase II and III trials?



For the Phase I trial, we looked for well-educated people who will grasp the science, who can give informed consent. They are role models, so other people will say, later on: if the doctors are doing this, it must be alright.

Going to the next level is really going to be a challenge. We will need to have education targeting women. Maybe we can mobilize communities to see this as something that both men and women can do for the epidemic.

What is your lower age limit for volunteers?

Now it's 18. But many people are not happy with 18. They say it is so young. Yes, they are young, but they are having sex and getting infected.

Other people say, what about including adolescents? This would open a whole different set of issues. How do you get consent? Who would give consent? If you ask the parents, they would say, yes I would want to know if my child was involved in this. Even with contraceptives, parents say they want to know if their daughters are using contraceptives. The daughters say, it is none of your business.

The other question is, would a vaccine make adolescents feel that all is well and they can go on with risk behaviors? Adolescents think they are invincible, that nothing can happen to them.

I would leave the age limit at 18, because it

continued on 8 ▶

◀ **MBORI-NGACHA INTERVIEW** *continued from 7*

avoids the issues of minors and trials. Maybe you would miss something, though, because people say there is a little bit of a biological basis that young women are at greater risk of acquisition. The vaginal canal is not fully developed, they get STDs, they don't have access to treatment. So they have increased vulnerability.

Can a 16-year old get contraception in Kenya without parental permission?

We are just beginning to put into place youth-friendly centers where you find peers to talk to and staff who offer you services without trying to "convert" you. When young women went to traditional family planning clinics they would find someone the age of their mother who would probably say, "What does a nice young girl like you want contraceptives for?" Many youth just go to a pharmacy and buy the pill across the counter.

At our teaching hospital we have a walk-in clinic for adolescents. There are a couple of others, but not many.

How many people know their HIV status?

Very few. It's only now that counseling and testing is becoming a big thing. At our walk-in clinic, people can get information about AIDS, they can be tested. In the rural areas, the government is committed to scaling up. Work elsewhere tells us that knowing your status translates into very good prevention strategies, because many people who are negative then change their risk behaviors.

But the number of people? Low, low, low.

How much access is there to childhood vaccines in Kenya, and how much uptake?

We have places in Kenya where the EPI (Extended Program for Immunization) is very successful—people go there and have their babies immunized. Then there are places where they don't.

We did some research on why women don't bring their children for measles immunization. It turned out that many people believe measles is a milestone, a rite of passage in a child's life—your child *should* have measles, because afterwards all will be well. So telling them that this vaccine prevents measles didn't have any pull. With this information the program could address those issues with the communities and highlight measles deaths, and emphasize that they are avoidable. Then the immunization rate came up.

What I'm saying is that we need to understand peoples' perceptions and expectations. Within Kenya there is a whole spectrum, from high to low vaccine coverage. Sometimes it's an access issue. But even where access is similar there are differences in vaccination rates. The social and cultural barriers have to be explored.

How widely available are treatments to reduce mother-to-child transmission used?

Mostly it's nevirapine at some pilot sites. The government is now trying to get nevirapine from the manufacturer so it's available country-wide.

But there are problems. Over half the women accept testing, but less than a third of those who test positive come back for the interventions. We are trying to understand this. Why would you not return, after going through this whole process, to benefit from what we promised in the beginning? When women come to the antenatal clinic, their agenda isn't to learn their HIV status. They want antenatal care. They may get tested, but if it comes out positive, many aren't ready to deal with that. Many are afraid.

We are doing this within a rural setting where the person providing care for you might be your relative, your neighbor, might be from your same village. So many women don't feel comfortable coming forth for the treatment.

I think we did it backwards, in a sense. We should have mobilized the communities so they would support a woman in using antiretrovirals for preventing transmission or for not breastfeeding her baby. Right now there isn't enough support. Her mother-in-law will ask and visitors will ask, and it will be very difficult for her to justify why she's not breastfeeding.

It's big, it's really big. The issues are more than just finding the right drug. Now we have something tangible we can do. But women are not using it.

When women in your clinics test positive, how many discuss it with their partners?

But this 40% has the potential to increase, because health providers have to change. It's not at all common to see men in prenatal clinics. The clinics need to invite men to come with their wives or partners; to initiate the dialog.

It helps when you introduce the topic of HIV testing to a couple together, and they take the test together. The outcome in terms of them taking up the intervention and the support the man gives the woman is much better this way. When you do it later it becomes very difficult for the woman.

What will be some of the issues for women around getting vaccinated, once there is an effective AIDS vaccine?

It's going to be very complex. When you go for a vaccine you could say one of two things: you either take risks yourself or you're implying that your partner does. We've seen this at our MTCT sites. If a woman says she would like to be tested, her partner may ask, what have you been up to? Why do you want to take the test? It's almost like an admission of something. Or are you accusing me? There is a no-win situation.

continued on 23 ▶

Closing in on Immune Protection in the Women of Pumwani

Can a Cohort of Kenyan Sex Workers Help Guide Us Towards an AIDS Vaccine?

BY RICHARD JEFFERYS

In the US, long-term studies of HIV-infected and high-risk people have mainly involved gay men—the group most heavily impacted in the epidemic's early years. But a continent away, in the Pumwani district of Nairobi, group of just over 100 women have become well-known to HIV researchers around the world by offering tantalizing evidence that the immune system can, in rare cases, fight off HIV.

The evidence derives from a cohort of women sex workers, established in 1984 by Elizabth Ngugi and colleagues from the University of Nairobi and the University of Manitoba for the purpose of studying STDs. Despite an estimated 60 or more unprotected exposures to HIV every year—one of the highest documented exposure rates in the world—just over 100 of the 2000 women enrolled in the cohort have tested negative for HIV infection for at least 3 years, and in some cases up to 15. Studies of these "highly exposed persistently seronegative" (HEPS, also sometimes referred to as "exposed seronegative" or ESN) women convinced many skeptics that immunological resistance to HIV—and by extension, an HIV vaccine—is possible.

Since the first description of this phenomenon by Canadian researcher Frank Plummer (at the 1993 International AIDS Conference in Berlin), the Human Immunology Unit of Oxford University in the UK has joined the Manitoba and Nairobi teams to conduct detailed immunological studies of these women. Their goal: to identify which immune responses protect the women against HIV, and to use that information to guide the design of preventive HIV vaccines.

Over the past few years, the Nairobi studies—along with those on other HEPS cohorts (see table, page 12) and on HIV-infected, long-term non-progressors—have been suggesting some answers. In the late 1990s they helped focus the AIDS vaccine field's attention on the importance of cellular immune responses in protection, especially the CD8+ killer T-cells (also called cytotoxic lymphocytes, or CTLs). These days, emphasis is on identifying the precise regions of HIV (called epitopes) that stimulate what appear to be protective responses, and on elucidating the roles of less well-characterized immune players, including CD4+ T-helper cells and mucosal responses, in resistance to HIV.

Alongside the science, the project was set up from the beginning to provide medical services for the women and frequent exchange with the research team. "The cohort is a partnership between the sex workers and the researchers," says Joshua Kimani, part of the team from the Department of Medical Microbiology at the University of Nairobi. "The partnership has worked over the years due to the monthly meetings we have with the sex workers' peer leaders. In these meetings issues related to poor follow up or any unhappiness with the service providers are ironed out." There is also an annual meeting between the researchers and the entire cohort. The research team provides free medical services, free condoms, covers hospitalization at the Kenyatta National Hospital and can assist with busfares and other expenses. Treatments available include those for STDs and the more easily managed opportunistic infections, but do not at present include expensive brand-name drugs such as the antifungal Diflucan and antiretrovirals.

The Origin of the Cohort

HIV testing in the Pumwani cohort began in 1985, when infectious disease specialist Plummer took what was intended to be a brief detour from Manitoba to join the STD project in Pumwani. Out of 600 women enrolled at the time, Plummer was dismayed to find that two-thirds tested positive for HIV. Shifting the focus of his work, he began to assess the factors associated with both seroconversion and, presciently, lack of seroconversion in the one-third of the women who tested HIV-negative. The startling observation reported in Berlin was that women remaining negative two years after starting sex work had only one-tenth the risk of subsequent seroconversion (over the following two years) than HIV-negative women newly joining the cohort. Furthermore, this apparent resistance to HIV infection was associated with certain class I HLA genes (see sidebar, page 11), suggesting a link to CTL responses.

Plummer's data caught the attention of Sarah Rowland-Jones, who had previously seen some cases of persistently seronegative women among sex workers in the Gambia. Joining up with the Manitoba and Nairobi investigators, Rowland-Jones and colleagues Tao Dong and Andrew McMichael analyzed blood samples from the HEPS women for evidence of HIV-specific CD8+ T-cell activity. Their results, published in late 1998 (*J Clin Invest.* 1998;102:1758), showed a strong association between the HEPS phenotype and the presence of HIV-specific CTLs direct-

continued on 10 ▶

◀ **WOMEN OF PUMWANI** *continued from 9*

ed against a broad range of HIV epitopes. This association was strengthened by later studies, while various explanations based on non-immune-factors, such as mutations in the CCR5 co-receptor gene, were excluded.

But then came a finding that initially seemed somewhat counterintuitive, according to Rowland-Jones: the level of the CTL responses in the HEPS women was as much as 10 times *lower* than in uninfected women. "That means that it's not enough to simply count T-cells," she says. Instead, it pointed the researchers towards a more qualitative analysis of the responding cells, for example in terms of their epitope specificity, breadth and functional properties.

Mucosal Responses

After the initial description of HIV-specific CTLs in blood, the researchers turned to analyzing the mucosal immune responses in the HEPS women. The first published study, led by new team member Rupert Kaul, reported the presence of HIV-specific IgA antibody in the genital tract of 16 out of 21 HEPS women compared to 5/19 HIV-infected women (*AIDS* 1999;13:23). Conversely, HIV-specific IgG antibody was absent from HEPS and present in all infected women. Working with Italian immunologist Mario Clerici to assess blood T-helper responses, the paper also reported evidence of Env-specific T-cells in 11/20 HEPS, but there was no correlation with mucosal IgA production.

The role of IgA was explored further in another collaboration, this time with Claudia DeVito and colleagues from the Karolinska Institute in Stockholm. The investigators designed a system to model the transfer of HIV across the human mucosal epithelium, then tested the ability of IgA isolated from the cervicovaginal fluid of HEPS women to block the transfer process (called transcytosis). Samples from six women were examined, and 3/6 reduced transcytosis of a primary clade B HIV isolate by more than two-thirds. The work suggests a mechanism by which IgA could contribute to protection at the mucosal surface, although the authors emphasize that other factors are probably also at play.

> *"HEPS women showed an enrichment of CTLs in the cervix relative to blood."*

In parallel to the IgA studies, the MRC group found evidence of HIV-specific CTL in the mucosa (*J Immunol.* 2000;164:1602). Examining cervical and blood samples, they found responses in 11/16 HEPS and 8/11 HIV-infected women using an ELISpot assay for interferon-gamma production. They also found that the HEPS women tended to have slightly higher responses in the cervix compared to blood, whereas infected women had significantly more HIV-specific CTL in blood compared with the cervix. This apparent enrichment of mucosal CTLs in the resistant women supports the idea that they play a role in protection from HIV.

Late Seroconversions

But as these studies were going on, unexpected findings were emerging: between 1996 and 2000, 11 of the 114 women who had met the working definition of "resistance" (>3 years sex work without seroconversion or a positive PCR) became HIV-infected and seroconverted (*J Clin Invest.* 2001;107:341). It took the researchers by surprise, since long-term seronegativity had appeared to be closely associated with a decreasing risk of infection. And it triggered an intense effort to find out what was going on.

It soon became clear that there was no obvious correlation between this "late seroconversion" and the presence or absence of CTLs in previous tests. "Half of the women who seroconverted had CTL [at earlier timepoints]," says Kaul. "We had looked at a couple of those women repeatedly and seen CTLs many times. So we were quite surprised and disappointed to see them seroconvert." An obvious possibility—that infecting viruses had "escape" mutations in regions targeted by the women's CTL—was quickly ruled out.

An answer began to unfold when the search turned to the amount or type of the women's recent exposure to HIV. Their analysis showed that a reduction in sex work—either stopping for over two months or reducing the number of clients by more than two per day—was strongly, but not absolutely, associated with subsequent infection: 10 of the 11 seroconverters had reduced their exposure by these criteria, compared to 10 of the 22 persistently seronegative women. Analysis of six women in the latter group found that—rather than seroconverting when they resumed sex work—they showed a boosting of their HIV-specific CTL responses. "In those women we saw a general trend that when you take a break from sex work, the immune responses go away," says Kaul. "If you start sex work again, these responses often come back." But it's unclear why the responses return in some women while others become infected. Some possibilities: persistence of HIV-specific CTL below detectable levels in the HEPS women, differences between blood and mucosal responses, the precise nature of the HIV exposure after a break and immune responses not analyzed in the initial study, such as HIV-specific T-helper cells and/or HIV-specific IgA antibody.

The implication of these results, widely reported in the mainstream press, was that continuous exposure to HIV may be important to maintain resistance in at least some HEPS women. Whether this would also apply to vaccines is unclear. The Oxford group point out two possibilities. One is that ongoing stimulation with HIV antigens is required, either through periodic vaccine boosters or through the use of vaccine strategies employing persistent antigen. Alternatively, vaccine-induced responses established prior to any HIV exposure (as opposed to immunity induced by live virus) might show a very different dynamic.

To look more closely for correlates of late seroconversion versus continued resistance, Kaul is now

involved in a prospective study, which will monitor a broad range of immune parameters. "We'll try and get women to come see us before they go on a break, so that we can look for HIV-specific responses at that time. Then we'll try to get them to see us as soon as they return, before they've started sex work again, so we can see what's happened to those immune responses." In addition to monitoring CTL from the blood, the researchers will also follow mucosal responses, while Keith Fowke from the University of Manitoba will study the CD4+ T-helper responses (see below).

The nature of the infecting virus in late seroconverters is also coming under the microscope. Some scientists have hypothesized that the resistant women have a latent, undetectable HIV infection, and that the late overt infections could represent an escape of this virus from immune control. "It would not at all surprise me," says Rowland-Jones. She's enlisted the help of Bette Korber from the Los Alamos National Laboratory and Harold Burger from the University of Albany to apply "molecular clock" techniques to date the viral isolates found in late seroconverters. "They plan to sequence virus to try and find out if it is an old Nairobi virus," reports Rowland-Jones. "Although this can't answer the question definitively, it might provide suggestive evidence of a latent infection."

The Search for "Resistant" CTL Epitopes

Another major focus of the current work is to identify the CTL epitopes associated with resistance. In the first set of data to emerge from this work (*J Clin Invest.* 2001;107:1303), the researchers report some striking differences. Looking at CTL responses to a panel of 54 known epitopes (restricted by 21 different HLA molecules), they found that HEPS women showed strong responses to four epitopes that were very rarely immunodominant in infected women—two in Pol and two in p24-Gag. They also found that infected women responded most strongly to epitopes recognized only rarely, or not at all, by the HEPS group. Of the seven late seroconverters evaluated in the study, five showed a switch from the HEPS pattern of epitope responses towards that of infected women and/or the complete loss of responses to the "resistant" epitope.

Another striking observation was that all four epitopes showing differences between HEPS and infected women are restricted by HLA alleles known to be associated epidemiologically with HIV resistance in the Nairobi cohort (A2, A24, A*6802, B14 and B18), suggesting that the effect of these HLA types is related to their greater likelihood of generating CTL responses to a repertoire of more protective epitopes.

The study represents a first step in identifying "resistance" epitopes, but there is more work ahead—particularly given the information gap revealed when the researchers use whole HIV proteins, rather than known epitopes, to measure T-cell responses. "We see a number of women who don't respond to a panel of CTL epitopes, but do respond to Env or Gag," says Kaul. "So there are probably some epitopes within those genes that haven't been mapped yet."

T-helper Responses in ESN Women

Not all the HLA alleles associated with protection in HEPS women belong to class I, the system for presenting epitopes to CD8+ T-cells. A comprehensive analysis by Kelly MacDonald's group from the University of Toronto (*J Infect Dis.* 2001;183:503) revealed a highly significant link with the class II allele HLA DRB*01, suggesting an important role for CD4+ T-helper responses in mediating resistance. "This points to the fact that there's a multifactorial immune response," says Keith Fowke, who has taken on the task of analyzing helper responses in the Nairobi cohort. "To ignore the T-helper response would be a mistake."

continued on 14 ▶

HLA Genes and Immunity

The Human Leukocyte Antigen (HLA) system is, in some respects, the immunological equivalent of a sophisticated alarm system. HLA molecules are produced within human cells, and act as receptacles for fragments of cellular or foreign (e.g. viral) proteins. The HLA molecules then display these fragments (known as peptides) on the outside of the cell; a single cell is typically adorned with several hundred thousand different HLA-peptide complexes. This process allows circulating T-cells to survey the HLA-peptide complexes for signs of any foreign peptides that might indicate the presence of a pathogen.

HLA molecules are divided into two major classes (I and II), which are recognized by different subsets of T-cells. The CD8 molecule on CD8+ T-cells interacts with class I HLA molecules. Likewise, the CD4 molecule on CD4+ T-helper cells interacts class II molecules. In both cases, the peptide associated with the HLA molecule is recognized by a structure on the T-cell called a T-cell receptor (TCR).

The critical aspect of the HLA system for immunity is that both class I and II molecules come in hundreds of different versions, dependent on the HLA genes inherited from our parents. The precise shape and size of an HLA molecule governs its ability to associate with a diverse array of peptides and present them to T-cells. HLA molecules thus exert a profound influence on the body's ability to mount a broad and effective T-cell response to any given pathogen.

Each individual has over 40 different genes that encode HLA molecules. The class I genes are divided into different regions (or loci), with the most important being HLA-A, -B and –C. The major class II genes are HLA-DP, -DQ and -DR. There are many variations of these genes in the human population and thus many variant HLA molecules. The different versions of the genes are known as alleles, and a complex classification system is used to characterize the specific HLA alleles that an individual has inherited The known alleles are numbered, for example, as in HLA-A2 or HLA-DR5. Analysis of the HEPS women has identified several class I and II alleles associated with resistance (see main article).

— R.J.

Studies on Exposed Seronegative Cohorts

The Nairobi ESN women are not the only group of exposed seronegative individuals being followed prospectively. Similar examples of possible HIV resistance have been reported from other cohorts, which typically fall into one of three categories: commercial sex workers (CSW) (who are usually exposed to many different HIV strains), "serodiscordant" couples (wherein one partner is HIV-positive, the other negative), and perinatally exposed infants (see article on page 8).

Recently published or presented findings from CSW and serodiscordant couple cohorts are listed in the tables below and to the right.

Location	Investigators	Type of cohort	Source of information (journal or conference)	Data published or presented
Northern Thailand (Chiang Mai and Lamphun)	Chiang Mai HEPS Working Group	Female sex workers	*J Infect Dis*.1999;179: 5	-- Criteria: >2yrs sex work, >2 STDs, HIV-1 seronegative. -- Resistance associated with HLA B18. -- Env-specific IgA detected in the cervix of 6/13 ESN women.
Addis Ababa, Ethiopia	Ethio-Netherlands AIDS Research Project	Female sex workers	*AIDS Res Hum Retroviruses* 2001;17:433	-- Criteria: >5yrs sex work, HIV-1 seronegative. -- No evidence for role of certain non-immune factors in HIV resistance (co-receptor polymorphism, co-receptor expression levels, beta-chemokine production and cellular resistance to HIV infection in vitro).
Abidjan, Côte d'Ivoire	Institute of Tropical Medicine, Belgium	Female sex workers	Int Conf. AIDS 2000, abstract # WePeA3995[1]	-- Some evidence of decreased CXCR4 expression and increased beta-chemokine production in both ESN and HIV+ FSW.
Chiang Rai, Thailand	Thai Ministry of Public Health and US CDC	Female sex workers	*AIDS Res Hum Retroviruses* 2001;17:719 Int Conf. AIDS 1998, abstracts #599/31120 & 603/31127[2] Conf. Retroviruses and Opportunistic Infections 1999 abstract # 83[3]	-- Criteria: >3yrs sex work, HSV-2 & syphillis+, HIV-1 seronegative. -- Resistance associated with HLA-A11. -- CTL responses to HLA-A-matched subtype E peptides (most commonly to Nef, also to Pol, Gag, Env) detected in 4/7 ESN. -- ESNs tended to have higher frequencies of polymorphisms in both the CCR5 promoter region and the gene for the chemokine SDF-1 (both associated with slower progression in HIV+ individuals) and higher spontaneous RANTES production by PBMC. -- Novel CD4/CD14-associated HIV suppressive activity detected in the blood.

1. *Int Conf AIDS 2000: the XIII International AIDS Conference, Durban, South Africa, 9-14 July 2000. http://www.iac2000.org*

2. *Int Conf AIDS 1998: the XII World AIDS Conference, Geneva, Switzerland, 28 June-3 July,1998. http://gateway.nlm.nih.gov/gw/cmd*

3. *Conf. on Retroviruses and Opportunistic Infections*
1999 Chicago, 31 January-4 February
2000 San Francisco, 30 January-4 February
2001 Chicago, 4-8 February
http://www.retroconference.org

Location	Investigators	Type of cohort	Source of information (journal or conference)	Data published or presented
Chiang Mai, Thailand	Thai Couples HEPS Group	Serodiscordant couples	Conf. on Retroviruses and Opportunistic Infections 2001, abstract # 51[3]	-- ESNs from a cohort of women married to HIV-infected men were compared to wives who became infected. Factors that did not differ between the two groups: HLA class I alleles, CCR5 D32 genotype and presence of SLP1 (an HIV-inhibiting protein) in the cervicovaginal lavage. -- Husbands' samples showed no differences in seminal viral load, HIV co-receptor use or defects in viral accessory genes. -- More than half the ESNs showed HIV-specific T-cell responses. -- ESNs showed CD14-associated HIV suppressive activity.
London, UK	Imperial College School of Medicine (St. Mary's, London) and MRC Human Immunology Unit, John Radcliffe Hospital (Oxford, UK)	Serodiscordant couples	Conf. on Retroviruses and Opportunistic Infections 2001, abstract # 52 & 53[3]	-- No mutations in CCR5 co- receptor gene or its promoter region. -- HIV-specific CD8 T cells detected in 8/8 ESN partners. -- Responses to whole HIV proteins but not known peptides, suggesting that responses are directed at unmapped CTL epitopes. -- Gag-specific CD4 T-cell responses detected in 3/5 ESN tested.
New Jersey, USA	Gladstone Institute/ Heterosexual AIDS Transmission Study (HATS)	Serodiscordant couples	Conf. on Retroviruses and Opportunistic Infections 2000, abstract # 595[3]	-- 13/16 ESN women showed ELISPOT responses to at least one antigen tested (Gag, Pol, Nef and Env); 9/16 responded to multiple antigens. -- 5/16 showed responses at multiple time points.
Lusaka, Zambia	University of North Carolina	Serodiscordant couples	Int. Conf. AIDS 2000, abstract # WeOrA596	-- Responded to whole HIV proteins (Gag, Env, Pol) -- HIV-specific CTL detected in 7/37 ESN.
Seattle, Washington USA	University of Washington School of Medicine and Fred Hutchinson Cancer Research Center	Serodiscordant couples	*J Infect Dis* 1999; 179: 548-57	-- 13/36 ESN showed HIV-specific CTL. -- 4/36 had lymphoproliferative responses (to p24, gp160 or gp120) detected in 4/36. -- 1/36 homozygous for the CCR5 D32 mutation, 10/36 heterozygous. -- HIV-specific CTL more commonly detected in individuals with wild-type CCR5 genotype.

◀ **HIV VACCINES AND BREAST MILK TRANSMISSION** *continued from 6*

breastfeeding transmission in exposed babies. This means it could take less time and a smaller sample size to determine a vaccine's effect.

But in practice, easy answers may be hard to come by. That's because a neonatal vaccine would not be a stand-alone intervention. Other strategies, such as antiretrovirals and immune-boosters, will be needed to provide coverage right after birth, before the immune responses kick in. And veterans of the neonatal vaccine field like Jack Lambert, are some of the staunchest supporters of the ARV-based approach.

"If an infant is born in Africa and we want to do vaccine studies, we can't say, 'We don't know if antiretrovirals work [in breastfeeding prophylaxis].' It's not proven for health-care workers either," he says. "I think we should have the same vision for infants as for health-care workers and build a vaccine strategy on top of that."

Other researchers and advocates suggest that treating women, at least for the duration of pregnancy and breastfeeding, could have a profound effect on rates of MTCT, as well as providing a foundation for more widespread adult antiretroviral treatment.

Pragmatists counter that while these strategies may be the best, they're not necessarily feasible right now. "We haven't really managed to get a two-tablet regimen out of Kampala," says Laura Guay, who points out that Ugandan efforts to provide NVP nationwide to HIV-positive pregnant women are proceeding by inches, rather than leaps and bounds. Human resources are the major bottleneck, according to Saul Onyango, the Ministry of Health official in charge of MTCT efforts He points out that training counselors, nurses and physicians takes time and money that's been slow in coming.

The fledgling field of neonatal vaccine research will face these and other tough issues. Will it be ethical to test neonatal vaccine efficacy if ARV prophylaxis proves effective? If not, will it be enough to measure immunogenicity? How will successive generations of trials address the issue of treating women, especially in light of research by Ruth Nduati suggesting that infants of HIV-positive women are more likely to die, regardless of their own serostatus? It's all part of an expanding vision for MTCT—one which incorporates many long-term threats to infant mortality. "If you keep the mother healthy, you keep the baby healthy—so you build your argument," says Lambert, who adds that vaccine studies can help with the implementation of proven interventions, like NVP. "We should go ahead with vaccine research—and take our known successes further." ◆

◀ **WOMEN OF PUMWANI** *continued from 11*

Fowke was recently lead author on the first published report to look at both HIV-specific T-helper and CTL responses in the HEPS women from the Nairobi cohort (*Immunol Cell Biol.* 2000;78: 586). This study detected T-helper responses in 7/17 HEPS women using an assay for IL-2 production in response to five Env peptides. Fowke's team then carried out both helper and CTL assays on samples from 15 women, and found a statistically significant link between the presence of T-helper responses and CTL. "The data is suggesting that it's important to have not only CTL but good help," notes Fowke. This observation is consistent with basic immunology work in animal models, demonstrating a key role for virus-specific T-helper cells in generating and maintaining effective CTL responses.

To clarify the role of CD4+ T-cells in protection, Fowke's group is using ELISpot assays to conduct a broader analysis of responses in the HEPS women. Although a significant amount of CTL epitope data is available there is a dearth of defined class II-restricted T-helper epitopes, one that Fowke aims to address by mapping the responses using clade A and clade A/D recombinant viruses. Another priority for the Manitoba team is investigating HIV-specific T-helper activity in the mucosa, which has never been studied in the cohort (or any other exposed seronegative individuals to date), due to the difficulty of obtaining samples with sufficient numbers of cells.

Shaping Vaccine Design

The presence of apparent immunity in the HEPS women, and its association with T-cell responses in the absence of antibody, have strongly influenced the thinking of AIDS vaccine designers—an influence readily evidenced by a new crop of candidates that aim to induce cellular immune responses to HIV. Several vaccines based on this strategy have shown promise in recent monkey studies, including those of Emory University researcher Harriet Robinson, Harvard's Norman Letvin and Merck & Co, Inc. (see *IAVI Report*, Feb/Mar 2000). As more is learned about the protective responses in the women of Pumwani, that knowledge is likely to continue guiding vaccine developers towards the types of responses to target and the HIV epitopes that can best induce them.

Results from the collaborative studies of the Oxford, Manitoba and Nairobi teams are also being passed on to vaccine designers Tomas Hanke and Andrew McMichael in Oxford, whose first generation DNA/MVA constructs are currently in IAVI-sponsored Phase I human trials in Oxford and Nairobi. Later generations of this vaccine will draw on information gleaned from the continuing work with these women. ◆

POSSIBLY, A VACCINE AGAINST HERPES – BUT FOR WOMEN ONLY?

By Patricia Kahn

After years without success in efforts to make a vaccine against herpes simplex virus type 2 (HSV-2), which causes chronic bouts of painful genital sores—and is present in over 20% of US adults—last year finally brought some progress. Results from two Phase III trials showed that a vaccine developed by SmithKline Beecham (now GlaxoSmithKline, or GSK) appears to offer some protection against disease. But the good news contained a shocker: it worked only in women—the first report of a vaccine that is effective in one gender only.

The vaccine, called Simplirix®, contains a recombinant form of the HSV-2 envelope glycoprotein, D_2, together with a new adjuvant called SBAS4. The two double-blind, multi-center trials collectively enrolled 2714 uninfected people from heterosexual serodiscordant couples (with one uninfected and one HSV-2-infected person). In the first study, which ran from 1995 to 1999, all 847 subjects (268 women) were seronegative for both HSV-2 and the closely-related HSV-1 virus, which causes fever sores in the mouth; the second study, begun later with 1867 participants (720 women), included participants with HSV-1. Volunteers were immunized three times (0, 1 and 6 months) and followed for 19 months, with monitoring for HSV-2 infection and clinical signs of genital herpes disease (GHD).

In both trials, the vaccine proved to be 73-74% effective against GHD in HSV-1-negative women, although it did not consistently prevent infection; effectiveness against infection was 48% in the first study and 39% in the second. But it showed no efficacy in men or in women infected with HSV-1. Transmission of GHD in the placebo group was about 11%, compared with 3% in vaccinees. The study did not determine whether protected women shed virus, and therefore whether they still transmit.

Coming after other (sometimes similar) HSV-2 candidate vaccines failed to show efficacy, Moncel Slaoui, GSK senior vice president for business and new product development, attributes this vaccine's partial success to the adjuvant. SBAS4 contains two components: QS21, which is known from other studies to enhance antibody responses (and was modified in this formulation to reduce its strong reactogenicity) and components from bacterial cell walls, which stimulate Th1-type responses, including cellular immunity. "We think that antibody responses are not enough, and that's why we use an adjuvant which induces cellular responses," he says. Slaoui adds that the adjuvant has proven safe in thousands of people, including those in an ongoing malaria vaccine trial in the Gambia, and that it will be used in GSK's planned Phase I trial of its protein-based HIV vaccine.

But there are no explanations for the apparent gender difference in efficacy. Vaccinated men and women both made HSV-2-specific antibodies, including the IgG form commonly associated with Th1-type responses; there were no direct measurements of T-cells or mucosal responses. Nor did preclinical studies yield any insight, since these were carried out exclusively in female guinea pigs so that HSV could be introduced into the genital tract more easily than in males.

Turning to speculation, Spotswood Spruance of the University of Utah, a principal investigator of one of the trials, offers a few. HSV-2-transmission to women initially exposes virus to the vaginal secretions, where it is accessible to immune players such as IgA and IgG antibodies, whereas men are probably exposed through tiny breaks in the skin of the penis that bring virus directly into contact with cells it can infect. Alternatively, the explanation could relate to broader immunological differences, such as those behind women's far greater tendency to develop autoimmune diseases. Slaoui's speculation is that the gender difference could reflect the speed and ease with which immune T-cells home to the genital mucosa in females versus males immediately after HSV-2 infection.

Some answers may come from a new trial of Simplirix® slated to begin next year. This time the study will involve a more general population of young people, rather than serodiscordant couples, and will include enough women to be statistically powered for a licensable result. Collaborators in academic groups will analyze the immune responses, says Slaoui, and hopefully give some insight into the mechanisms at work—which may then suggest a strategy for making an HSV-2 vaccine that also works in men. "We don't know what that strategy would be, but the present vaccine gives us a toehold," says Spruance.

What are the chances of a similar outcome for an HIV vaccine? While any answer is only a guess, it can't be ruled out, says Slaoui. On the other hand, he notes that the hepatitis B vaccine—the only licensed vaccine against a sexually transmitted disease—is used for both men and women and, like HIV (but not HSV), the virus causes systemic disease after being acquired via the genital tract. But he also says that GSK will be prepared for a gender difference with its HIV vaccines. "We will set up our trials for HIV so we can detect efficacy in women only," he says. ◆

> *"An experimental vaccine shows 73% efficacy in women but none in men."*

High-Risk Women at a New York Phase III Trial Site

AN INTERVIEW WITH

Pamela Brown-Peterside

A short distance - but a world away - from the bustle of Manhattan, the South Bronx is home to one of two "Project Achieve" HIV prevention research sites, this one focused on high-risk women. Run as a collaboration between the New York Blood Center's epidemiology lab and New York City's Department of Health, the site conducts vaccine and preparedness studies, along with trials of behavioral interventions and microbicides, in cohorts of mostly poor, minority women. It is participating in VaxGen's 5,400-person North American/European efficacy trial, with an enrollment of 59 (out of 309) high-risk women in the otherwise gay male study population.

Pamela Brown-Peterside, the site's Nigerian-born director, has a Ph.D. from the Columbia School of Public Health. Here she speaks with the* IAVI Report *about the challenges of maintaining a cohort of women who live a precarious existence at the fringes of society, and about the HIV risks they face.

How did the cohort get started?

We began here in 1995 as part of Project Achieve's HIV prevention work. The project's community advisory board had advocated strongly for a women's component to the effort, although we had to push hard to get it funded. That led us here to the South Bronx, which is one of the highest HIV prevalence areas in New York [about 4%], and to a collaboration with the New York City Department of Health.

We started with two small cohorts. One was a 'first-generation' vaccine preparedness study (VPS) called "Achieve" that looked at issues of recruiting and retaining high-risk women but didn't include vaccine education. The other, called VPS I, was a cohort through HIVNET [the NIH-sponsored HIV vaccine trials network], and it had a vaccine education component.

The original plan was to recruit from the STD [sexually transmitted diseases] clinic in this building. But that didn't provide the steady stream of participants we expected, for several reasons. So we moved to a street outreach approach, which is what pulled most of the women in.

Our retention rate for those initial cohorts was pretty horrendous. We were very new to what it would take to involve women in these studies. In some ways, we were naive about their life circumstances—for instance, that it was a very transient population. If somebody's phone was disconnected or they moved, we lost them.

We learned a lot. But still, we had the feeling we weren't getting the highest risk women. It's a trade-off: for the sake of retention you want more settled people, but they may not be the highest risk group. HIVNET then also became interested in a higher-risk cohort. That led to VPS II.

What was different the second time around?

We narrowed the criteria for enrollment. To be eligible, women had to have a current male sex partner who was either HIV-positive or an injecting drug user, or within the past year they must have either exchanged sex for money or drugs, had five or more male sex partners or had an STD. At this site, we didn't include any women who were themselves injecting drugs, because we chose to focus on sexual transmission.

We also hired a former VPS I volunteer to do outreach for us. She was actively using crack when she first came here, but her involvement in the study and the relationship she developed with her counselor turned her life around. She is now doing very, very well.

People trusted her. Many of the women who enrolled in VPS II came through "friends" or "friends of friends." She was really able to tap into that network, because she was part of it. Our target for the cohort was 150, and we over-enrolled-we got 164 people in a very short space of time.

This time we had tremendous success with retention. We had learned that to hold onto people, we needed the names and addresses of at least two contacts at the start of the study. It also helps that our retention specialist has been on board from the beginning. Six months into VPS II we had retained 96% of the women and 92% after 12 months — this in a cohort of very poor women, where the majority report using crack.

HIV incidence in VPS II was 1.18%. So somehow we are still not tapping into the highest risk women. That might be partly a function of age. The women we see are in their early to mid-thirties. We're not reaching them in their early twenties or late teens, where the incidence is increasing most rapidly.

What would it take to bring in more younger women?

We will have to change the way we recruit. We would have to identify places in the area where

young women hang out—bars, malls, shopping areas, movie houses—and go there at different times, including evenings. And we would need young women who are part of that network to do the recruiting.

How did you move from the preparedness study to a Phase III trial?

The VPS II created a group of women who were very ready to be involved in an actual vaccine trial. They had been exposed to information on what a vaccine trial involved and had become comfortable with the idea of taking part in the effort to find an HIV vaccine.

We also put some ads for volunteers in the newspaper and pulled in some women who had not been in VPS II. They ended up being quite different. Usually more educated. Some were health care professionals.

What are the lives of the women like?

There are two issues that stand out most. The first is their poverty. Almost 90% reported less than $12,000 income in the past year. Most are unemployed and on welfare, on Medicaid. Many have children who they support alone. Most have not completed high school. Some are homeless, or nearly so, or have kids in the foster care system.

The other issue is substance abuse. Two-thirds of the women in VPS II said they used crack within the past year.

In terms of numbers of sexual partners, over half reported having five or more partners in the last year. We see a lot of 'serial monogamy,' as well as a number of women with a main partner and several other partners who give them money or crack, in a casual way - it's not a formally negotiated transaction. Very few are actively engaged in sex work.

Violence is also an issue. In the VPS II cohort, 30% of the women said they were beaten by their partner within the last year, and two-thirds had seen someone else beaten up. When you're talking about the women trying to negotiate condom use, this is the context you need to look at.

What reasons do the volunteers give for joining the trial?

The woman we hired to do outreach for VPS II openly admits that she first came into our office because she heard in the street that she could make ten dollars, which is what we paid people for visits in VPS I. That's all she was interested in, because she was on crack. We've had other women, especially those in the vaccine preparedness studies, also tell us that they initially came because of the reimbursement.

Once they're here, a big part of what keeps them is the counselors. There were women who said to Debbie [Debbie Lucy, senior counselor in the trial], I'll join the vaccine trial because I want to keep coming back to see you.

But, given the intensive screening process for the trial, it's unlikely that people are motivated to enroll by the reimbursement. Many join an actual trial because the epidemic has touched them in a profound way, and volunteering is a way of giving back.

How do the counselors work with volunteers living under such difficult circumstances?

One of the things we recognized early on was the need for a case management component to the work. The women have so many needs in terms of getting their welfare cases reopened, or their Medicaid hooked up. Housing issues. Violence. Getting into drug treatment or mental health services. The counselors spend a lot of time on all of this. We deal with some of those needs by having a pretty good referral list, and following up during subsequent visits.

These women don't seem to really talk or get much support even from their women friends. They're all just trying to survive.

So it's very important for them to come here and have a counselor who gets to know them, who does risk-reduction counseling, and so on.

The Project Achieve's South Bronx team. From left to right: Kathleen Bremer Covitz, nurse practitioner; Evelyn Rivera, retention specialist; Denise Goodman, community relations coordinator; Debbie Lucy, senior counselor; Pamela Brown-Peterside, project director; Beryl Koblin, principal investigator. Not pictured: Verna Robertson, nurse practitioner; Regina Wiley, outreach worker.

Even though our focus is HIV prevention, our counseling covers the gamut in terms of their needs. I think that's a big reason why women keep coming back. The women really connect with the counselors here.

And we have really made an effort to make this a woman-friendly space, and to give people a positive experience here. It's very

continued on 23 ▶

Gender, HIV Transmission and Vaccines: A Moving Target

BY ANNE-CHRISTINE D'ADESKY

Emerging data suggest that HIV transmission, acquisition, and disease progression are influenced by gender.

In many countries around the world, the rate of HIV infection in women is rising faster than in any another group. In sub-Saharan Africa, where over 70% of the world's HIV-positive people live, women made up about 55% of those living with the virus at the end of 1999, according to UNAIDS, and young women (ages 15-24) in the hardest-hit countries were up to three times more likely to be infected than males of the same age. In the US, women accounted for 23% of all new AIDS cases in 1999, compared to only 7% in 1986.

This vulnerability is deeply rooted in social and behavioral issues like poverty, gender power dynamics, and in many regions of the world, a decreasing age of sexual debut. But are there also biological factors at work? Do gender differences affect HIV transmission, as an intriguing new study suggests? If so, how? What about differences in immune responses? And what does this mean for vaccine development and other preventions?

For the past few years, such questions have sparked a growing debate among some HIV clinicians and researchers, as new data emerges to suggest that HIV can affect women and men differently. Several studies show that women's initial viral load levels are up to 50% lower than men's, though both progress to AIDS at the same rate—an important finding when it comes to antiretroviral therapy and clinical management of HIV disease. It also raises questions for vaccine developers, who increasingly view viral load as an indicator of efficacy for candidate vaccines that do not prevent infection but block progression to AIDS.

Turning to immunology, there are some known (but mostly ill-understood) gender differences which could influence responses to HIV. For starters, the female immune system has evolved to perform a delicate balancing act: it must protect the highly exposed genital tract from all manner of infections, but at the same time refrain from attacking immunologically foreign sperm; nor does it attack a fetus. It's also long been observed that women are at much greater risk than men for developing autoimmune diseases, although only recently has the field begun to tackle why this is so.

For HIV, several studies have linked local immune responses in the genital tract to protection in women—for example, in highly exposed, persistently seronegative sex workers (see article, page 9). It is also becoming clear that HIV in the genital compartment evolves independently from that in blood, with local immune dynamics influencing the process, and therefore that studies of HIV in blood alone may not give a complete picture.

But even acknowledging such differences, there has been no evidence that they can actually translate into different outcomes for any vaccine—that is, until last year's reports of data from two Phase III trials of an experimental vaccine against herpes simplex virus-2 (HSV-2) (see article, page 15). The startling results: signs of efficacy in women—the first apparent success for a candidate HSV-2 vaccine—but none at all in men.

Transmission and viral load

New data on gender differences and viral load come from a recently published 5-1/2 year study of 317 heterosexual HIV-serodiscordant couples in Zambia (*AIDS Res. Hum. Retroviruses* 2001;17: 901). In the study, a team from the University of Alabama at Birmingham (UAB) led by Susan Allen and Grace Aldrovandi used epidemiological linkage to confirm "true transmission pairs" and concluded that, as plasma viral loads increased, individuals were more likely to pass on HIV to their uninfected partners.

But surprisingly, the link appeared much stronger in women than in men, in whom the association between high viral load and transmission was deemed only "weakly predictive." For instance, women with viral loads above 100,000 were nearly six times more likely to pass on the virus than women with less than 10,000 copies, while for men the differential was less than two. The gender difference was especially pronounced at low viral loads—the most important range in terms of assessing how vaccines that reduce viral load will affect transmission rates. While only 9% of women with viral loads less than 10,000 passed on the virus, 24% of the men in this range transmitted (although the numbers of individuals were quite small).

"I think everyone suspected viral load to be a factor, and no one had looked at gender differences," states Sten Vermund, a UAB researcher who worked on the Zambian study. "Our finding was not at all subtle. It was very strong and consistent."

The Zambian study followed on the heels of a similar analysis in serodiscordant couples in Rakai, Uganda (*N Engl J Med* 2000; 342:921). There, a high level of HIV in the blood was also linked to an increasing risk of transmission. But the Rakai study reported no significant differences between men and women.

Why the discrepancy? It could well lie in the different study designs, says Thomas Quinn of John Hopkins University in Baltimore, who was lead author of the Rakai paper. Quinn was quick to praise the Zambian study as "fascinating and important." But he cautioned that his group didn't analyze viral load data above 100,000 copies, so a true comparison of the two studies is impossible. "We just lumped them all together above 50,000 copies," he said. The Rakai

team also included fewer women in its study population, and they measured viral load in serum, not plasma, which is slightly lower. Besides these factors, says Quinn, "I could probably list ten variables, all of which play some modifying role in sexual transmission." He ticks off a few: condom usage, circumcision, viral load levels in blood versus genital secretions, age, presence of other STDs, HIV clade, duration of partnership, and the presence of acute seroconversion in the infected person. And of course, immune factors—the big gray area.

Clinicians are familiar with the variability. "We know cases of women who have undetectable plasma viral loads who transmit, and there are certainly cases of women with very high plasma loads who do not transmit," says Kathleen Squires, an HIV clinician at the University of Southern California. Similar observations have been made for men. And transmission is clearly also affected by factors influencing HIV acquisition in the uninfected partner, as perhaps best illustrated by the cohorts of female sex workers who remain seronegative despite repeated, intensive exposure to many different HIV-infected male partners.

From Viral Load to Infectivity

Recognizing that there is more to infectivity than viral load in the infected person's blood, several lines of research are trying to identify additional factors that affect both transmissibility and acquisition—information that could be key for understanding how vaccines which lower viral load may affect transmission overall.

An obvious place to look is in the genital compartments, where sexual transmission of HIV takes place. Susan Cu-Uvin is an Associate Professor of Obstetrics and Gynecology at Brown University who is studying HIV and immunity in the female genital tract. Says Cu-Uvin: "The majority view is that the higher the plasma viral load, the higher your genital tract viral load. But there are outliers. It's not everybody. Depending on the method you use to measure, and on the subcompartment of the genital tract, you will find that—at least in my work—up to 50% of the time, the genital tract viral load is higher than in plasma. So I think we can say they are separate compartments." Others have reported different figures of discordance in these reservoirs, but come to the same conclusion. Adds Cu-Uvin: "If you only study the plasma, you make assumptions that might not reflect what is going on and where the [transmission] action is taking place. Because you don't have sex in your plasma—HIV is a mucosal infection."

But even viral load in the directly-transmitting compartment is not the whole story, says USC's Squires. "Even if you look at maternal-fetal transmission studies, it's clear that there is not a one-to-one correlation" between high viral load and transmission, she says. To understand infectivity, "we need to look at genital tract viral load and do a head-to -head comparison between men and women. Given the right circumstances, both can be infected with HIV; it's not a gender-selective infectious disease. But what happens once they are infected may be different. Viral load in the genital tract is not the end of the story, either."

Backing these statements are a flurry of data showing that distinct viruses evolve in plasma versus the rectum, vagina, semen or breast milk. For example, Ted Ellerbrock of the Centers for Disease Control (Atlanta) recently found that plasma and vaginal secretions of HIV-positive women contained different viral populations, based both on genotype and on the emergence of drug-resistant subpopulations (*J Infec Dis* 2001;184:28). In his study, only 2% of the HIV in vaginal secretions were common to blood. In another example, Carmen Zorrilla (University of Puerto Rico) reported different patterns of HIV resistance in paired plasma versus vaginal swabs taken from HIV-positive women on HAART therapy (Abstract No. 719, 8th Conference on Retroviruses and Opportunistic Infections).

HIV Acquisition and Immune Responses

Immune components in the genital tract are also likely to be important modulators of infectivity, although the evidence so far is mostly suggestive rather than definitive. The data come partly from studies of SIV in primates and HIV in mice (see *IAVI Report*, May-June 2001, page 1). In these models, HIV-specific CD8+ T-cells (cytotoxic T-lymphocytes, or CTLs) have emerged as key players in mucosal defenses to HIV, and CD4+ T-helper cells also have a role. Other clues come from cohorts of commercial sex workers who do not seroconvert despite repeated exposure to the virus. High HIV-specific CTL levels in the genital tract may be contributing to this viral resistance, alongside humoral responses like secretory IgA (sIgA) (see article, page 9).

At the Fearing Laboratory of Brigham and Women's' Hospital in Boston, Deborah Anderson is studying HIV in the male genital tract and blood. Working closely with Cu-Uvin, she has found that the most important mediators of T-cell responses are present at the urethra near the opening of the penis. "The mucosal underside of foreskin is a very important area to look at," she says. Some key questions: how mucosal responses compare with those in the vaginal mucosa and whether they can be enhanced with a vaccine.

That raises the practical question of how to do these studies. The big obstacles are a lack of standardized diagnostic tools to sample viral load in the genital tract, and quality control of samples. "I can do a viral load on a finger prick with just a filter paper and a drop of blood," says Quinn. "It's well standardized. The way genital tract samples are collected is much more difficult."

Part of the problem is variability. "The female tract is so dynamic that it's different from today to tomorrow," states Cu-Uvin, noting that hormones, menstrual cycle and STDs all affect the delicate bal-

continued on 20 ▶

ance of this microenvironment. The other part is the collection method. "If you use (cervical) lavage, you're diluting your sample," she says. "With the snow strip, the volume is so small you can't do additional studies. With the cytobrush, 97% of samples are contaminated with blood. We just haven't found the best way of doing it [in women]. In men, you masturbate, that's it." Another issue in women is ensuring that the collected virus reflects the woman's own infection rather than that from the semen of an HIV-positive sex partner.

In the meantime, some insights can be gained from *in vitro* studies. For instance, Anderson's laboratory and others have succeeded in culturing pieces of vaginal mucosa to study antibody transport at the molecular level. Other groups have developed laboratory models of sexual transmission to track how and where HIV breaches the mucosa of the human genital tract and then infects underlying cells, and what immune activation takes place. As Anderson notes, "We need *in vitro* systems to complement these *in vivo* observations, which are so difficult to interpret."

Spotlighting the role of hormones

As they look to explain gender effects in HIV, researchers usually find themselves turning to hormones. "Why do women have lower plasma viral loads?" asks UAB's Vermund rhetorically. "Nobody has a clue. But women and men's makeup are nearly identical, and the key differences are hormones [and anatomy]. Are there unknown hormonal influences that either up-regulate HIV in men or down-regulate it in women? That could have therapeutic and other implications."

As more questions pile up, HIV researchers are hampered by an overall lack of knowledge about basic reproductive biology and mucosal immunology. "There is not a whole lot of data available on what happens in the 'normal' vaginal tract," says Squires. "You can look at infections in pregnancy and endometriosis and those kinds of models as a corollary to what happens in the genital tract when a woman has an infection. But a big problem is that the hormonal milieu [in these circumstances] is so different."

Compared to the peripheral immune system in the blood, the human mucosal system consists of a complex, integrated network of tissues, lymphoid and mucous-membrane cells, plus effector molecules such as IgA antibodies, cytokines, chemokines and their receptors—all of which act in concert with innate host factors such as defensins. Both cell-mediated and antibody responses occur in the mucosa, where adhesion molecules allow immune cells to "home" to sites of infection. Studies of sexual transmission show that HIV targets the cervix and that compared to men, the cervix offers a larger target cell area for the virus than the head of a penis. But key features that distinguish the male and female immune system are sex hormones and regulatory cytokines that control the level of antibodies like secretory IgA and IgG in vaginal fluid and tissues.

As Squires points out, pregnancy is a time when the immune system in a woman must somehow allow immunologically distinct fetal cells to survive. Sex hormones like estrogen and progesterone are thought to direct that response. They're also the fingered culprit in studies on why women are so prone to autoimmune disorders. In his textbook review of genital tract immunity, William Kutteh notes, "The composition of genital secretions (including IgG and IgA levels) is dependent on hormonal and inflammatory factors" (*Mucosal Immunology*, 1999). He and others report that the endocervix and ectocervix have a very high accumulation of immunoglobulin-forming cells that produce predominately sIgA antibodies. Other HIV and SIV transmission studies in female mice and primates show that these viruses initially target the cervix, including the lamina propria (just under the epithelium), before spreading to other sites.

Hormones can also affect immune dynamics at the epithelial surface of the cervix. At the Tulane University Primate Center, Preston Marx has reported that progesterone thins the epithelial cell layer of the cervix in primates, allowing SIV to more easily infect target cells. This monkey model may shed light on what is seen in young girls, where the lining of the cervix is known to be thinner than in older women and thickens with the onset of puberty. Such biological factors may play a role in making teenage girls more susceptible than older women to HIV infection. They might also help explain observations that women on birth control pills have a somewhat elevated risk of HIV acquisition, although a biological (as opposed to behavioral) basis for this has not yet been conclusively shown.

"The progesterone link is so interesting," says Squires. "I think it's short-sighted of us not to try to figure out what happening because we might be able to utilize this."

Evaluating Vaccines

Taken together, what do these findings mean for vaccine research? With so many questions still unanswered, the clearest implication is that gender should be firmly on the agenda as clinical trials begin to evaluate how HIV vaccines that lower viral load affect disease progression and transmission. Will these vaccines have different clinical benefits in men and women, given the gender differences surrounding viral load? And will lower viral load levels affect infectiousness in men and women differently?

With most of the current HIV vaccine candidates unable to block infection but apparently working by lowering virus levels, these become critical questions. The more answers we have, and the more information on factors contributing to infectivity—including viral load, STDs, male circumcision status and mucosal responses—the easier it may be to answer these questions and guide future vaccine design and testing. ◆

Women, HIV Risk and Vaccines in a Rural South African Community

BY MICHELLE ROTCHFORD GALLOWAY

In the remote rural regions of South Africa, young women constitute the highest risk group for HIV infection, as they do throughout much of the continent. Over the past several years, one such location—a tribal ward called Hlabisa (pronounced "shlabisa")—has launched intensive vaccine preparedness efforts, under the auspices of South Africa's Medical Research Council (MRC) and NIAID's HIV Vaccine Trials Network (HVTN). Here we describe some aspects of the work relevant to women's risk and their possible willingness to participate in Phase III trials, based on interviews with Quarraisha Abdool Karim and Janet Fröhlich, and data supplied by Salim Abdool Karim.

Quarraisha Abdool Karim, an epidemiologist and molecular biologist, was the original director of the site and was involved early on in mobilizing the community. She has recently moved to the University of Natal. Fröhlich comes from a background of grassroots HIV/AIDS work among community organizations and is MRC research site manager at Hlabisa (and presently Project Director of the Vaccine Preparedness Study).

Starting out from the coastal city of Durban, it takes three hours of strenuous driving—the last part on steep, winding, dirt roads—to reach the tribal ward of Hlabisa. The village has no formal addresses, so visitors are guided to their destination with instructions such as, "turn left where the old tree used to be." Its people survive on subsistence farming and income sent home by men working hundreds of miles away as migrant laborers in South Africa's mines and large urban industries.

That's where the explosive AIDS epidemic in this region begins. It continues when the men bring HIV home to their wives or girlfriends, and is catalyzed by the extremely high number of relationships between girls in their teens and men in their 30's or older.

The result is that young women between the ages of 15-19 years old are up to three times more likely than males the same age to become infected with HIV according to S. Abdool Karim—a gender disparity that has existed since the early days of the region's AIDS epidemic. Seroprevalence in women attending prenatal clinics, determined by anonymous HIV testing, has shown a rise from 4.2% in 1992 to 34% in 1999, with incidence rates rising from 2.3% per annum in 1993 to 15% per annum in 1999 (as measured by detuned ELISA tests). In the 25-29 year age group, a staggering 45% of the women were HIV-positive, and their death rate is more than double that of men the same age.

From Vulnerability to Participation

Hlabisa's involvement in vaccine testing is an outgrowth of the prevention and epidemiological studies started there in the early 1990's by David Wilkinson, then superintendent of the district hospital, and Salim Abdool-Karim, then with the MRC and now at the University of Natal in Durban. In 1997, largely as a result of that groundwork, the Hlabisa and Durban groups received NIH funding to begin developing the infrastructure and cohorts needed for Phase III trials of HIV vaccines, as one site within a network of trial sites then called HIVNET. (The network was revamped in 2000 and is now the HVTN).

With this solid funding, the researchers were able to begin real vaccine preparedness activities. One was an intensified surveillance effort, which among other things provided more precise data on the disproportionately high risk to young women. Another is a vaccine preparedness study, now in full swing, in which community educators will visit 2500 households by the end of this year, collecting data from all consenting household members (ages 15-54 for women and 15-70 for men) on a broad range of HIV-related questions. Starting with standard demographic and health information, the survey then asks about the labor migration patterns of household breadwinners, sexual behaviors relevant to HIV, willingness to participate in vaccine trials and factors influencing that decision. After pre-HIV test counseling, blood is drawn and arrangements made for participants to receive the results, along with further counseling, at the study clinic.

As the researchers begin to analyze early results, the outlines of some gender-related differences are beginning to emerge.

Temporal trends in the age-specific prevalence of HIV infection in prenatal clinic attenders in Hlabisa

Age Group	1992	1995	1998
20-24	6.9%	21.1%	39.3%
25-29	2.7%	18.8%	36.4%
30-34	1.4%	15.0%	23.4%
35-39	0.0%	3.4%	23.0%

Source: Wilkinson D, Abdool Karim SS, Williams B, Gouws E. High HIV incidence and prevalence among young women in rural South Africa: developing a cohort for intervention Trials. J Acquir Immune Defic Syndr. *2000;23: 405.*

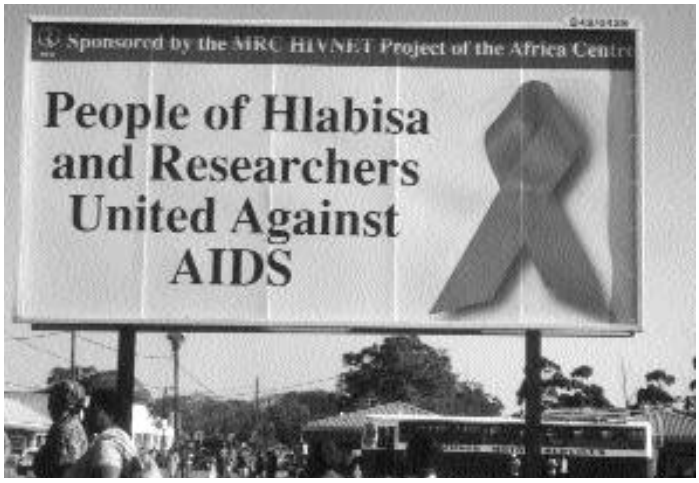
continued on 22 ▶

◀ **EMERGING DATA** *continued from 21*

"Our preliminary data suggest that about two-thirds of the women interviewed indicated willingness to participate in HIV vaccine trials, compared to a third of the men," says Quarraisha Abdool Karim.

Overall, both men and women cite altruistic reasons for their willingness to participate, and their belief that a vaccine brings hope to their community. But women were much more likely than men to cite the need to protect themselves against HIV as another reason to participate—a motivation that will need to be addressed in the pre-enrollment knowledge-building, but which suggests that "women have a keener perception of their risk of acquiring HIV," says Abdool Karim.

But she adds that it is too early to tell whether this general willingness will translate into a concrete decision to volunteer when a Phase III trial actually gets underway. While this will partly depend on the requirements of the specific trial, the broader issue is that of women's lower status and degree of autonomy in making decisions—an issue that emerged even in the 18-person Phase I HIV vaccine trial now ongoing in Nairobi (see interview with Dorothy Mbori-Ngacha, page 7).



"Constitutionally, women have a right to give individual consent, but in reality it may not always work," says Fröhlich. "This is part of the knowledge-building that will have to occur prior to recruitment. Rural women will need guidance as to what their rights are and will need to understand that they have their own voice in decision-making."

Fröhlich says there are also differences in the types of logistical support it will take to involve women versus men into trials. With women closely tied to home through their responsibility for household, farming work and childcare, finding time to attend a study clinic located as far as 25 kilometers away along poor roads would be extremely difficult for some, and therefore mobile clinics may be necessary to secure their participation. In contrast, unemployed men tend to congregate in the middle of the village, making them easier to access, while men with jobs are often working far away from Hlabisa in the urban centers.

These men's long absences raise the somewhat unusual issue of the difficulties that could arise in including an equal proportion of men in vaccine efficacy trials. Follow-up visits to the clinic would require either that the men make more frequent trips home, or that trials are somehow set up to accommodate their absences. One possibility, suggests Fröhlich, is "a collaborative multi-site model in which participating migrant laborers could be monitored at different trial sites." While that would clearly require a new level of logistics and organization, she is hopeful that a solution can be found. "Migrant workers shouldn't be discriminated against in [trial] inclusion criteria because migrant labor is a reality of the South African situation," she says.

At this point, says Abdool Karim, discussions are still on very general terms. But once a definite product is ready to move into Phase III—probably in 5-7 years time—decisions will become more concrete.

In the meantime, as the crisis of the sick and dying continues to worsen, the focus in Hlabisa is on finding feasible ways to provide more care. About 80% of the patients at the Hlabisa District Hospital are AIDS patients, most of them young women. But the 95-bed hospital is completely overwhelmed in terms of staff, space and lack of medicines (and too far away for many Hlabisa Ward residents to access), which has led to growing use of mobile clinics that visit the remote areas in each ward about once in six weeks. Home-based care is also on the rise through efforts of the partnership between the research project's Community Advisory Board (CAB) and the hospital HIV/AIDS and TB program. The CAB is also working on palliative care initiatives and support for orphans and disrupted families. For the short-term, MRC researchers are launching two small trials of natural medicines—*Aloe ferox* for treating AIDS-related diarrhea and *Sutherlandia microphulla* for cachyxia—for which there are "good anecdotal findings" of some effectiveness, along with widespread community acceptance, says Fröhlich. On the prevention front, a key goal is bringing about wider HIV testing and counseling, now limited both by the hospital's understaffing and "a reluctance in the community to go for testing because of a lack of privacy—in the hospital, everybody knows everybody," she says.

So for now, the community of Hlabisa struggles on amid the devastation of the epidemic, especially on its young women—and the prospect of a future vaccine that has so strongly engaged the ward which now calls itself the "Village of Hope." ◆

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◀ **MBORI-NGACHA INTERVIEW** *continued from 8*

I think the key is to say that this is good for the family. In MTCT we say in Kenya that pregnant women who go for HIV testing are good women because they want to protect the child. There are many issues with that approach. It promotes knowing her status just for the child, not because it is a good thing for the woman herself. But we used the feedback we got—that we as a society value our children and believe we should protect them.

In the vaccine arena, I think it will be similar, where you say vaccines are important to protect men and women for future generations. It has to be really thought through. People are always in denial about their own risk, or their children's risk. ◆

◀ **BROWN-PETERSIDE INTERVIEW** *continued from 17*

hard to measure this piece of it, but we're thinking about possibilities—scientifically you want to be able to point and say, this is contributing this much.

What other issues do you face in the trial that are specific to women?

Because of these issues that come with poverty—especially transient housing and homelessness—we discovered that we need to be in touch with the women frequently. So, even though the vaccine trial only calls for visits once every six months, we make sure we contact them midway through that window, to make sure that they're still where we think they are.

The other issue that women bring, which we rarely think about in HIV vaccine trials, pregnancy. We're working with high-risk women, and most of them use condoms as their primary mode of contraception—sporadically. As is very common, they tend to use condoms with secondary partners, but not their primary partner.

So we have had to deal with the issue of pregnancy during the trial. I think that's an issue which really needs to be taken into consideration.

What do you do?

Before people enroll, we say that if they're making a commitment to the study, they shouldn't intend to become pregnant during the study period. That's one of the reasons why women choose not to participate, because they think they might want to become pregnant.

If they do become pregnant, we stop their immunizations but still follow them through the rest of the trial.

But the fact that women will become pregnant obviously has implications for sample size and other aspects of protocol design.

What does your experience so far tell you about the prospects of getting larger numbers of high-risk women into Phase III trials in the future?

I'm a strong advocate of making sure that women are included in HIV vaccine trials, and not just considered an afterthought. I hope that concerns about recruitment and retention, which have been issues in the past, won't be used as reasons to exclude high-risk women from vaccine trials.

It's different, and more difficult, I suspect, than recruiting men who have sex with men. We know those differences well, because we have a men's site. In general the men have multiple modes of contact—home phone, work phone, e-mail, cell phone. It tends to be a more stable group. Here we work with volunteers who don't even have a phone.

But we've demonstrated here that you *can* recruit and retain women. So has Richard Novak's team at the University of Illiniois in Chicago, which has a larger high-risk women's cohort. But it's much more labor-intensive to follow this type of semi-rooted or transient population. And it requires more resources. We need a full-time retention person just to hang onto the cohort. But the resources are not always forthcoming to do longitudinal studies, and do them well.

What else can be done to keep women involved?

It's hard when there are so few women. This trial has 300 women out of 5,400 volunteers in total. It's clear from the sample sizes that this is an MSM trial and that it's not geared to women.

To keep high-risk women involved, we need to recognize that they have different needs than men. They may need case management services, help with transportation for appointments—even if this means simply covering the cost of a bus ride to and from the clinic. They also need ongoing counseling, for example, around the issues of pregnancy and contraception. And there are other basic things, such as having a clinic space that is welcoming to children, who often accompany women on their visits.

In a larger sense, we've often had the feeling that the needs and concerns of women are not taken seriously in HIV prevention more generally. It's been a struggle. As we move into the future, I hope that women don't continue to be on the margins of this work but that we can take our rightful place as equal partners in this effort. ◆

IAVI Report

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vaccine BRIEFS

Presidential AIDS Advisory Council Presses On, Renewing Call for Vaccine Support

At its July meeting, the U.S. Presidential Advisory Council on HIV/AIDS renewed its call for the U.S. government to pursue a comprehensive, aggressive strategy to promote HIV vaccine development. The Council extended its prior recommendation on strengthening public-private partnerships to emphasize the need for policy reforms that will promote developing country access to safe, effective vaccines as soon as they become available. In particular, the Council recommended tax credits for companies engaged in vaccine R&D, tiered pricing of vaccines, and the availability of a global purchase and distribution mechanism.

Ending speculation that the Council might be terminated under the new administration, senior White House officials confirmed that President Bush planned to continue the advisory group. However, the exact membership and future function of the group remain unclear. New members are expected to be named as terms expire for some of those appointed by former President Clinton. The remaining members have staggered terms and will cycle off the Council in 2002 and 2003.

Early in its existence, the Council staked out a leadership role on vaccines, successfully urging President Clinton to set a target date for an HIV vaccine. The Council has held extensive hearings on various vaccine-related topics. One hearing that generated particular attention featured the late Jonathan Mann, who testified that human rights principles demanded removal of obstacles to development of a vaccine.

Free Online Journal Access for Developing Countries

Six major publishing houses have announced plans to provide free electronic access to their scientific journals to medical schools, research laboratories and government health departments in low-income countries. The project is a collaboration with the World Health Organization (WHO) and initially aims to serve about 600 institutions, mostly in Africa. The journals will be available through a special WHO-administered internet portal called the Health InterNetwork. The publishers involved are Elsevier, Wolters-Kluwer, Blackwell, Harcourt General, Springer-Verlag and John Wiley & Sons, a list that covers many vaccine- and AIDS-related journals.

The British Medical Journal (BMJ) Group is also offering free access to its journals for anyone in counties defined by the World Bank as Low Income Economies. Potential subscribers can follow standard subscription procedures for any BMJ group journal and the subscription system will automatically recognize the location, allowing free access to those in qualifying countries. A full list of journals is available on the BMJ group website at: http://www.bmjpg.com/.

Allovaccination as an AIDS Vaccine Strategy

At the May meeting on Vaccines & Immunotherapy in Puerto Rico, Jonathan Leith of the University of Toronto presented data from the first human study of allovaccination as a potential AIDS vaccine strategy. The study involved women receiving leukocyte immunotherapy for the prevention of recurrent spontaneous abortions.

Leukocyte immunotherapy is carried out by immunizing women with irradiated peripheral blood mononuclear cells (PBMC) from their male partner, in the hope of preventing immunological rejection of the developing fetus during pregnancy. The HIV study examined whether antibodies directed against foreign HLA molecules could neutralize virus *in vitro*. The rationale for this approach is that HIV incorporates HLA molecules into its envelope when budding from infected cells, and therefore might also be targeted by the anti-HLA antibodies.

Leith reported that two of the seven women studied made IgG antibodies specific for the HLA molecules of their partner. In one individual, these antibodies were directed against only class I HLA molecules, while the other made antibodies to both class I and class II. He then presented data from Tom Matthews of Duke University (Durham, North Carolina), who tested the ability of these antibodies to neutralize HIV grown in the partner's T-lymphocytes. Matthews found that antibodies to class I alone were unable to neutralize the virus, but that antibodies targeting both class I and class II neutralized both a laboratory-adapted and a primary HIV isolate. Class II is important, said Leith, an observation bolstered by recent *in vitro* studies showing preferential incorporation of class II versus class I molecules by HIV (J. Virol. 75:6173, 2001).

While only one of seven women made a neutralizing antibody response, Leith stressed the preliminary nature of the study, noting that the leukocyte immunotherapy involved only a single dose of irradiated PBMC without any adjuvant. Reviewing the potential of allovaccination for HIV, he also pointed out that the use of at least five different HLA class II alleles would be necessary to cover more than 95% of the possible HLA types in humans, and that such a vaccine strategy could be employed only in areas where organ transplantation is rare (since anti-HLA immunization would greatly increase the risk of rejection). The University of Toronto group, which is headed by Kelly MacDonald, intends to pursue the approach in the SIV model.