

AIDS VACCINE RESEARCH

HIV Natural Resistance Field Finally Overcomes Resistance

People who fend off HIV despite repeated exposures may have genetic or immunologic factors working for them that can help guide AIDS vaccine research

WINNIPEG, CANADA—In 1989, Stuart Shapiro and his pregnant wife, Awuor, went to the obstetrician to learn whether she carried the gene for sickle cell anemia common in people from Kenya, her home country. The results shocked the young couple: No, she did not have the gene, but the doctor said Awuor was infected with HIV. “With one sentence, our world fell apart,” said Shapiro, who had been with Awuor for 7 years. The doctor suggested that they consider terminating the pregnancy, but Awuor carried to term and gave birth to a daughter.

To their great relief, Stuart was not infected, and Awuor did not transmit the virus to their daughter. In part because he wanted inside information about the latest anti-HIV drugs to help Awuor, Stuart, a retrovirologist, went to work for the U.S. Food and Drug Administration. But Awuor’s health steadily deteriorated, and in 1996, she died from AIDS. As the years passed, Stuart became increasingly convinced that studies of *his* blood might help spare others from the fate his wife had suffered. He had, after all, likely been exposed to the virus repeatedly. If some combination of genetic and immunologic factors had protected him, unraveling them might inform researchers struggling to develop an AIDS vaccine and other biomedical preventives.

From 15 to 17 November, the first-ever meeting on natural immunity to HIV was held in this small city on the Canadian prairie, and Shapiro was one of 100 scientists from around the world who attended. Now a program officer at the U.S. National Institute of Allergy and Infectious Diseases (NIAID), Shapiro oversees a \$50 million annual grant to an academic consortium called the Center for HIV/AIDS Vaccine Immunology (CHAVI). At the meeting, he not only represented NIAID but also spoke for other people who have dodged the HIV bullet and donated their blood for study, and who are frustrated that the field remains so dis-

jointed and underappreciated. “I want them to study the phenomenon extensively,” said Shapiro. “I don’t want them to leave any stone unturned.”

Just as Edward Jenner used milkmaids who did not develop smallpox to design a vaccine that ultimately eradicated that disease, researchers here had high hopes that their findings will help in designing an effective AIDS vaccine. But so far, few tangible advances have come from studying people like Shapiro, despite years of efforts.

Dozens of studies have been examining men and women who sell sex but remain uninfected, and similarly, clients of sex workers, “discordant couples” like Shapiro and his wife, hemophiliacs who received tainted lots of blood, babies like Shapiro’s daughter, injecting drug users, health care workers who accidentally poked themselves with contaminated needles, and men who have sex with many male partners. But all too often the leads point in contradictory directions, in part because investigators use different assays to probe their samples, and there is little coordination among them. Many labs also use wildly varying criteria to decide who qualifies as HIV-resistant, making it difficult to sort out which study subjects were truly exposed and uninfected, were exposed and have an occult infection, or were never exposed in the first place. “If we are getting anything out of this meeting, it will be

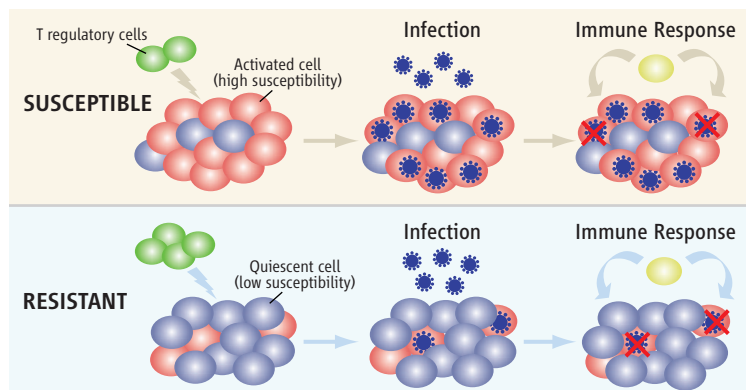


Healthy relationship. Stuart and Akinyi Shapiro lost a wife and mother to AIDS, but neither became infected. Why?

to come to some agreement about the people we’re studying and the degree of risk they have faced,” said Frank Plummer, the meeting’s organizer and head of Canada’s National Microbiology Laboratory, which is based here and has an \$8.3 million grant from the Bill and Melinda Gates Foundation to study natural immunity. “And it’s quite possible there are different mechanisms at work in different groups.”

For more than 20 years, Plummer has followed a group of sex workers in a Nairobi slum that includes more than 100 women who by all odds should have become infected but are not. With a nudge from the Gates Foundation, Plummer and his team invited colleagues who have similar cohorts to Winnipeg to see whether they could all collaborate. “Before, this was seen as a freakish phenomenon,” said Mario Clerici, an immunologist at the University of Milan in Italy who helped pioneer the field with Gene Shearer of the U.S. National Cancer Institute (NCI). “Gene and I were totally in left field: Nobody believed natural immunity to HIV existed. Now opinion leaders are jumping into it.”

Studies of the Nairobi sex workers continue to provide clues about protection. Keith Fowke, a microbiologist at the University of Manitoba here



Quiet type. People resistant to HIV may have fewer “activated” cells, presenting the virus fewer targets and making it easier to clear the few infections that do occur.

who works with Plummer, explained that they have focused on the 5% of more than 3000 women in their cohort who on sensitive PCR blood tests remain uninfected by HIV after at least 3 years of selling sex. Some of the women deemed resistant have later become infected, leading Fowke to stress that the protection against HIV is not absolute. Yet he is certain that these resistant women have genetic and immune responses that helped them thwart the virus. Fowke, Plummer, and their co-workers at the University of Nairobi have homed in on the resistant sex workers' unusual CD4 white blood cells, which coordinate the immune response against HIV and are also its main target. Unlike people at low risk of HIV infections, CD4 cells from these women copy themselves vigorously—or “activate”—when exposed to pieces of the virus in test tube studies, indicating that they have confronted the enemy before and can quickly rev up the immune system to block infection. But these HIV-specific first responders make up a tiny fraction of the CD4 cell population. And the general CD4 cell population in the resistant women had unusually low levels of activation markers. This indicates that most of their CD4 cells are in a “resting,” or quiescent, state, save for those that are actively responding to HIV—and that may be key to their resistance.

Various cellular factors make it difficult for HIV to infect resting CD4 cells in the blood, forcing the virus to rely mostly on the activated ones to establish an infection. So smaller populations of activated CD4s mean fewer targets for HIV. In something of a paradox, activation is good when it's directed against HIV but bad when CD4s are turned on high for other reasons.

In support of their immune-quiescence hypothesis, Plummer, Fowke, and colleagues found that the resistant women had higher levels of regulatory T cells that directly tamp down activation. When Fowke's lab compared gene expression in CD4 cells in resistant women and uninfected controls, the resistant group had far fewer genes turned on high, also suggesting a quiescent state.

Fowke suspects that the reduced number of targets in immunologically quiescent people makes it harder for HIV to succeed because even if a few cells do become infected, the immune system has a better chance of snuffing out the fire before it spreads. It made a compelling story, but as often happens in this field, Clerici followed with a study in discordant heterosexuals in Milan that suggested activation *helped* people avoid HIV, putting a damper on the quiescence theory. “I have data that are absolutely the opposite,” apologized

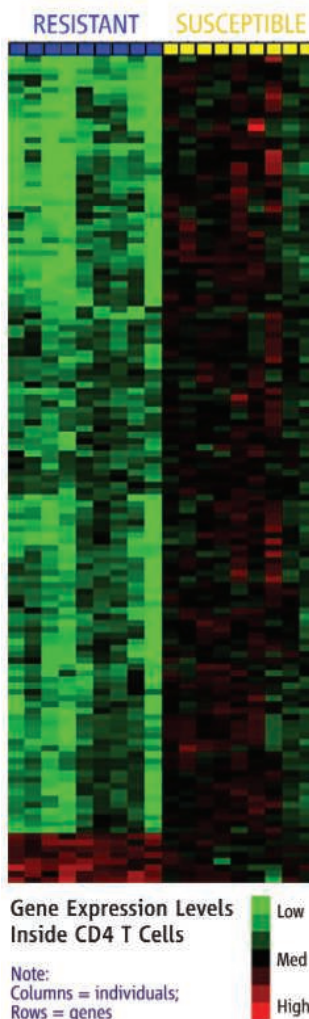
Clerici, who noted differences in the way they analyzed samples. “I don't know what to say.”

Other disparate findings presented here suggest that myriad factors contribute to HIV resistance. Activation is part of the refined adaptive immune system that remembers specific pathogens and mounts attacks when they reappear. Gianfranco Pancino of the Pasteur Institute in Paris linked protection in exposed injecting drug users in Vietnam to the more primitive innate immune system, showing that they had unusually high levels of natural killer cells—which are much less selective than adaptive immune actors like HIV-specific CD4s—to clear infections. Kristina Broliden and Klara Hasselrot of the Karolinska University Hospital in Stockholm found that uninfected men who had oral sex with long-term infected male partners developed powerful “neutralizing” HIV antibodies in saliva that might prevent infection by that route. Several genetic studies identified a handful of specific genes associated with protection that may turn on underappreciated immune responses.

Immunologist Michael Lederman of Case Western Reserve University in Cleveland, Ohio, found the often-conflicting leads sobering. “This is one of the most interesting and important problems that's facing HIV research today,” he said. “But I'm actually quite pessimistic that we're going to be able to sort this out.”

Lederman said he was not confident that the populations being studied were “clean enough” to fish out correlates of protection. “When we're talking about folks who are exposed through sexual exposure, quantifying the risk is kind of difficult,” he said, because there is no way to determine what HIV dose—if any—they have actually thwarted. So Lederman has turned to studying the carefully characterized groups of uninfected hemophiliacs who received contaminated lots of blood-clotting factors in the early 1980s. As

Gene Expression Profiling In HIV Resistance



Array of hope? Genes inside the immune cells of HIV-exposed, resistant sex workers in Kenya are not expressed as much (green) as those in susceptible, uninfected people.

a group led by NCI's James Goedert showed in 1994, just 5% of hemophiliacs in the United States and Europe who received moderate to high doses of contaminated clotting factor did not become infected. That makes this group of uninfected hemophiliacs the most unequivocally resistant people to study.

Lederman and co-workers compared stored blood samples of 36 HIV-uninfected hemophiliacs from this group with samples from healthy controls. They looked for differences in susceptibility to infection, neutralizing antibodies, and chemokines but found nothing notable. The team did discover, however, that their cells were less likely to be activated to copy themselves, supporting the Plummer group's findings. But Lederman remained circumspect: “Correlation does not confirm causality.”

Investigators working with CHAVI recently began intensive genetic and immunologic studies of blood samples from additional hemophiliacs who meet the same criteria. Shapiro says they hope to have 600 samples within the year, and they have already started to do genome-wide association studies and full-

genome sequencing of some individuals. CHAVI is also ramping up resistance studies in men who have sex with men, discordant couples, and mother-infant pairs.

By meeting's end, the researchers agreed to form a consortium to hammer out how they will share samples, standardize assays, and agree on definitions of HIV resistance. Although sorting wheat from chaff will remain challenging, one thing will not, says Shapiro: finding HIV-resistant people to volunteer for the studies. “We all wonder why we didn't get infected,” said Shapiro. “It's almost like being a Holocaust survivor. And if studying us can help bring an end to the epidemic, it can help us make some sense of our lives and the suffering we've seen and felt.”

—JON COHEN