AIDS Research Shifts to Immunity

Most studies have focused on how people get sick. Now a host of provocative new studies are zeroing in on why some people stay well—and the findings could hold lessons for vaccine development

A decade into the AIDS pandemic, two widely held and fundamental assumptions about the progress of this scourge may be falling by the wayside—with potentially profound implications.

For much of the 11 years since the disease was first recognized, the vast preponderance of epidemiologists, researchers, and clinicians have believed that, even though most infected people do not get sick for years, ultimately all who are infected will fall ill. Secondly, it has been the common wisdom, until quite recently, that repeated exposure to HIV through sex will virtually guarantee infection. Now comes a slew of provocative studies being conducted at many different medical centers worldwide that are uncovering immunologic clues that may help explain why some people who have been infected since as far back as the late 1970s have remained symptomless and why others, who repeatedly have met up with the virus, have remained uninfected.

As investigators begin to look hard at why some people stay healthy in the clutches of HIV, a basic shift in the focus of AIDS research is taking place: "For the past five, six years of this epidemic, people have been looking at correlates of [disease] progression," says Bonnie Mathieson of the Division of AIDS, a branch of the National Institute of Allergy and Infectious Diseases (NIAID). "Now they're finally starting to look at correlates of protection." And this new focus on immunity to HIV has already begun to make researchers question widely held assumptions about what an HIV vaccine needs to do.

Natural immunity: fighting off HIV

The optimal AIDS vaccine would teach the immune system how to stop HIV from establishing an infection. In the best case scenario, vaccine designers could study people who had naturally accomplished that feat and then mimic whatever their immune systems were doing. But because such people had been found, AIDS vaccine developers were forced to guess what, exactly, their preparations should do.

A few years ago, immunologist Gene Shearer of the National Cancer Institute (NCI) concluded that some people must have hardy enough immune responses to HIV to avoid infection. "When I learned from epidemiologists the number of people exposed but not infected," says Shearer, "I said it sounds to me more immunologic than the role of the dice."

Working with NCI immunologist Mario Clerici and Janis Giorgi of the University of California, Los Angeles, Shearer studied five men who reported having had unprotected, anally receptive sex with a known HIV-infected partner during the preceding 9 months. Yet none of these five men had antibodies to HIV. Repeated attempts to culture virus from their blood failed. What's more, all of them tested HIV-negative with the ultrasensitive polymerase chain reaction (PCR) assay.

That was interesting, but it might have been the case that, by chance, in their encounters these men actually hadn't been exposed to HIV. So the immunologists looked for evidence that these apparently uninfected men had once had HIV in their bodies. Though the humoral arm of the immune system—the one that makes antibodies—offered no clues, that was only half of the equation. The immune system also has "cell-mediated responses," which direct killer T cells, or cytotoxic T lymphocytes (CTLs), to eliminate cells infected with foreign invaders such as viruses.

To probe for clues of cell-mediated responses to previous HIV encounters, the investigators tapped into the immune system's "memory banks," coaxing these men's blood cells with four synthetic HIV-1 peptides. In each case, this probing stimulated production of the immune-system signal called interleukin-2 (IL-2)—which is precisely the cell-mediated response that would be expected in the face of an invader the system has seen before.

Perhaps, the NCI team reasoned, in these subjects a cell-mediated response had preceded the production of antibodies, clearing HIV before the virus could acquire a foothold. As the researchers conclude in a report of the study in the June *Journal of Infectious Diseases*, "It is possible they were exposed to HIV-1 or HIV-1 antigens at a level sufficient to prime T cell immunity but insufficient to induce antibody." If so, then the right T cell response might, by itself, derail HIV infection.

There are many caveats to this study, as the authors

STUDIES IN SURVIVING HIV

Researchers	Subjects	Findings
Mario Clerici and Gene Shearer, National Cancer Institute Janis Giorgi, University of California, Los Angeles	Uninfected homosexual men who have repeatedly had anally receptive sex with known infected partner	Uninfected men's peripheral blood mononuclear cells produced interleukin-2 when stimulated with HIV-1 envelope peptides, suggesting previous exposure and cell-mediated response
Fred Valentine and Mindell Seidlin, New York University Medical School	Uninfected heterosexual partners of infected people with whom they have had repeated unprotected sex	Uninfected partners' CD4 cells proliferate when exposed to HIV's envelope protein, suggesting previous exposure and cell-mediated response
David Ho, Aaron Diamond AIDS Research Center	Patient with acute HIV infection	Initial burst of virus appears to have been knocked down solely by cell-mediated response
Marshall Posner, Harvard Medical School	HIV-infected patients at various disease stages	Healthiest patients have rising titers of CD4 binding site neutralizing antibody
Susan Zolla-Pazner, New York University	HIV-infected patients at various disease stages	No correlation between disease and V3 neutralizing antibody titers
Jay Levy, University of California, San Francisco	Long-term survivors	CD8 cells especially adept at suppressing HIV replication, largely via unidentified soluble factor

stress. The HIV these men saw might not have been infectious. The IL-2 assay could be misleading. (In fact, a few controls in the study also reacted in this way to HIV peptides.) One of the five men followed during the 20-month study did later show antibodies and persistent infection, raising the possibility that he was infected all along and the virus was sequestered in, say, a lymph node. Then again, he may have continued his high-risk behavior until a putative defense was overwhelmed.

Despite the caveats, leading AIDS researchers don't dismiss the data. "I think it's interesting and should be pursued," says NIAID director Anthony Fauci. When asked whether he believes there are exposed, uninfected people, Fauci says, "There have to be. There absolutely have to be. There's no way that's not the case."

The NCI group is not the only research team grappling with this phenomenon. At New York University (NYU), Fred Valentine and Mindell Seidlin are struggling to understand the immune systems of 80 heterosexuals who are uninfected—as determined by antibody, PCR, and culture tests—even though they have had unprotected sex with infected partners. All the couples in the study (who are being compared to 80 couples in which both partners are infected) report having had unprotected sex at least 10 times—but the median is much higher: 500 times. "Overall, you'd expect these people were amply exposed," says Valentine.

Why aren't they infected? In a test similar to the IL-2 assay, the NYU team has mixed HIV envelope protein with key immune-system cells called CD4s (the very ones that HIV infects) from a few dozen of the uninfected partners to see whether those cells would proliferate—another indication that the immune system has seen the invader before. The CD4s did proliferate. "I don't know what to make of this except it begs the whole question: Can one be sensitized to making cellular responses without making antibody?" says Valentine. "I offer the data, not the conclusions."

But NIAID's Mathieson sets great store by such studies, because they are, for now, the only ones that offer data about people who might have completely fought off HIV. "Until we actually have vaccines protecting people in efficacy trials," she says, "I don't think we're going to have individuals who compare to these people."

After infection: preventing disease

But what if researchers get nowhere at actually blocking infection? That would make it more desirable to keep the virus, once established in a person's system, under control. And there are recent indications that the immune system may be able to keep the virus in check.

The search for humoral and cellular responses that might protect infected people has led David Ho, head of New York's Aaron Diamond AIDS Research Center, to home in on the very early period of infection—attempting to find out how the immune system handles the virus's initial assault, which it often seems to do quite deftly. During this "acute" phase of infection, the amount of HIV skyrockets to the levels seen in patients with full-blown AIDS. Then, in most cases, the viral load plummets. "That's without drugs," says Ho. "It's spontaneous. I happen to think that that is immune-response directed. That's consistent with our understanding of immune control of other viral diseases."

Again, as in the case of those who may be showing natural immunity, the cellular arm of the immune system could be the key. "Very, very preliminary evidence," says Ho, suggests that what is responsible for controlling HIV "is cell-mediated immunity." Ho and his colleagues recently did a detailed analysis of one patient with acute HIV infection and found, much to their surprise, that the initial burst of virus was significantly knocked down before they could detect either antibodies that could latch onto the virus and neutralize it or a type of killing known as antibody-dependent, cell-mediated cytotoxicity (ADCC). But the drop correlated with the appearance of CTLs, the killer lymphocytes that rid the body of infected cells and are a crucial part of the cell-mediated response. Ho now is looking at similar parameters in a few more patients with acute HIV infection.

That's a hopeful portent, but, for most people, fighting off the virus at the beginning of infection is not enough to prevent disease in the long run, and several researchers are focusing in on the people who harbor the virus but have not become ill. No one knows how many infected people—if any—have reached a permanent immunologic standoff with HIV. But researchers following large cohorts of infected people have found distinct groups of people who seem to be beating the odds.

Big Studies Set On AIDS Immunity

AIDS research in the United States seems to be undergoing a sea change, as researchers shift their focus from what causes disease to what causes immunity. But this research interest is so new that there are few large studies to back it up. Now, however, large immunological studies in four groups of "unique individuals" are on the runway and set for takeoff at the Multicenter AIDS Cohort Study (MACS).

The MACS is the flagship AIDS epidemiological project in the United States, an NIAID-funded effort that since 1985 has been following almost 5000 gay men to track the course of HIV infection. Recently, scientific leaders of the four different MACS sites—Baltimore, Chicago, Los Angeles, and Pittsburgh*—decided to send their studies off in a new direction. On 8 June they formalized plans to look closely at their cohorts of more than:

- 250 uninfected men who reported having unprotected, anally receptive sex with several partners (some of the partners were known to be HIV-infected).
- 40 "rapid progressors" who developed full-blown AIDS within five years of a documented infection.
- 50 infected men who have remained free from all AIDS-related diseases for more than 3 years, despite having counts of fewer than 200 CD4 cells. (The loss of these critical white blood cells is generally considered a hallmark of AIDS. Normally, people have counts of 800–1200 CD4s.)
- 50 infected men who, without treatment, have had a rise in their number of CD4 cells.

Each group is being carefully matched with controls. Specifically, these studies will use banked cells and serum, in addition to new samples, to try and tease out the immunologic, virologic, and hereditary factors that can explain protection and progression.

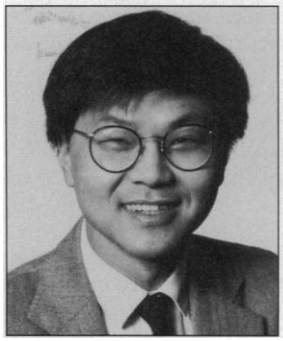
Epidemiologist Roger Detels of the University of California, Los Angeles, is heading the uninfected, high-risk study and believes the MACS researchers are mining a gem-filled vein. "We've tended to neglect the obvious, to not look at the issues surrounding infection itself," says Detels. "If there are factors associated with resistance to infection and we can characterize what those are...we may be able to effectively immunize people."

John Phair, a Northwestern University Medical School specialist in infectious disease who chairs the MACS's executive advisory committee, says these studies will be the major focus of the MACS for the next 18 months. "We think [the MACS] has evolved into a major resource," says Phair. "If we are clever, we can get some very useful information out of this."

–J.C.

*The MACS centers and their principal investigators are as follows: *Baltimore* Johns Hopkins School of Hygiene and Public Health, Alfred Saah; *Chicago* Howard Brown Memorial Clinic/ Northwestern University, John Phair; *Los Angeles* University of California, Los Angeles, Schools of Public Health and Medicine, Roger Detels; *Pittsburgh* University of Pittsburgh Graduate School of Public Health, Charles Rinaldo, Jr.

The San Francisco City Clinic Cohort, one of the oldest AIDS cohorts in the world, formed in the late 1970s for hepatitis B studies in homosexual men, has been following 516 infected people who have well-defined dates of seroconversion (the time they first showed HIV antibodies in their blood). On the average, these people develop AIDS symptoms within 10 years. But there is a group of 135 people who have been infected between 10 and 14 years who have never had an AIDS symptom. "We have some men who really do not seem to be getting sick," says Susan Buchbinder, who heads clinical studies in the cohort's research branch.



STEVE TURNER

Bonnie Mathieson *(above)*, David Ho.

Other studies are zeroing in, at the cellular level, on the question of why these people are staying well. At the University of Massachusetts, John Sullivan says they're following a patient who has been infected with HIV for 10 years yet apparently has an intact immune system. Though the man is seropositive, the researchers can only find the virus in him with a special, hypersensitive "boosted" PCR technique. A check of the regulatory genes from his viral samples showed those genes were intact, suggesting that the virus was indeed pathogenic.

Sullivan doesn't know why this man isn't ill, but he thinks "escape mutant" theory might provide a partial answer. While most people seem to be infected initially with only a single strain of HIV, over time this hypermutating virus creates a swarm of different strains. Even if the immune response can handle the first strain, it must keep expanding its repertoire constantly to combat mutant HIVs. Eventually, says NCI's Peter Nara, who has described an escape mutant model in detail, the virus may "outstrip the capacity of the immune system" to respond. Sullivan wonders whether his remarkably healthy patient has somehow prevented the virus from replicating—and hence mutating—as quickly as it does in most people, which might also provide clues to keeping other people healthy.

Over at the University of California, San Francisco, Jay Levy, a virologist who was one of the first researchers to isolate the AIDS virus, is following up on his own theory about what distinguishes long-term survivors. Levy is studying the immune systems of about 40 subjects, some of whom are with the San Francisco City Clinic Cohort. In these people, he finds that the T cells studded with a surface marker known as CD8 can strongly suppress HIV replication rates without killing infected cells. Levy believes the suppression is primarily the work of an unidentified "soluble factor" secreted by the CD8 cells—which are lead actors in the cell-mediated immune response—and into these patients' bloodstreams. "I don't know what it is, but I know what it isn't," says Levy, who has checked every known immune system factor for such effects—and come up with nothing.

All these studies—suggestive as they may be—are far from complete. Even so, they have already begun to offer some practical pointers for AIDS vaccine researchers. The most immediate lesson could be a shift toward greater emphasis on cell-mediated immunity, which almost all of these studies indicate plays a large role in keeping the healthy that way.

To date, many AIDS vaccine developers have relegated cell-mediated immunity to a backseat, banking on their ability to crank up production of neutralizing antibodies. They view these antibodies as crucial because they might, in theory, confer "sterilizing immunity," preventing HIV from infecting even a single cell of the body. Such total prevention is viewed as especially critical because HIV, like all retroviruses, integrates with genes of the host cell, where it can quietly hang out—possibly undetected by the immune system—only to surface years later.

That view makes sense. But, unfortunately, the evidence that neutralizing antibodies can protect a person from initial infection remains thin. Most work on neutralizing antibodies has focused on trying to raise antibodies against a specific portion of the viral envelope protein—the so-called V3 loop. Yet, save for a few influential chimpanzee experiments, precious little has been demonstrated in living organisms about the ability of V3 antibody to protect against infection.

Furthermore, attempting to correlate V3 antibody to protection from disease has been problematic. It is well known that people go on to develop full-blown AIDS even when they have relatively high levels of neutralizing antibody. And NYU immunologist Susan Zolla-Pazner looked at 29 patients at various disease stages and found no correlation with V3 antibody titers. "A number of studies have been very controversial and contradictory about whether neutralizing antibody correlated with progression to disease," says Zolla-Pazner. "There's no consensus." Still, Zolla-Pazner believes that sterilizing immunity is the goal, and her studies suggest that combining V3 with other neutralizing antibodies may just provide the necessary synergistic wallop.

As the cell-mediated data emerge from recent studies, however, some researchers are resetting their sights. "I think there has been an overemphasis on neutralizing-antibody work," says Aaron Diamond's Ho, whose lab does much neutralizing-antibody work. "The ideal vaccine would elicit good neutralizing antibodies and some cell-mediated immunity."

Others are even more emphatic. Polio vaccine developer Jonas Salk, who cofounded San Diego's Immune Response Corporation to design an AIDS vaccine, has long contended that cell-mediated—not humoral—immunity is the key. "The virus enters the host in a cell, whether the route is intravenous, seminal fluid, or vaginal secretions," says Salk. "It's in a cell. Therefore it's like a cancer cell, and the immune mechanism must be cell-mediated."

Lessons to be learned?

What practical effect will this gathering paradigm shift have on AIDS vaccine research? For one thing, an AIDS vaccine might need to lock a person in to what immunologist Peter Bretscher of the University of Saskatchewan calls a persistent cell-mediated state of immunity. Bretscher has shown over the past 20 years that repeated low doses of antigen can, as he says, "imprint" a mouse's immune system to turn on the cell-mediated arm and simultaneously shut off the humoral one. Bretscher suggests in an in-press *Immunology Today* paper that such a strategy may lead to an effective AIDS vaccine.

NIAID's Mathieson, however, thinks the lesson to be learned here is that there may never be a single correlate of protection, be it antibodies or cell-mediated immunity. "Everyone's looking for one correlate of immunity," says Mathieson. "In the end, that's not what it's going to be." Maybe a mix of cell-mediated and humoral responses will do the trick. Maybe an initial infection, followed by a strong cell-mediated response, is the answer. Maybe a more vigorous CTL response during acute infection or more potent neutralizing antibodies will slow down HIV's replication rate, reducing the viral "swarm" and prolonging the immune system's life. Maybe stimulating production of Jay Levy's soluble CD8 factor will prevent disease progression in chronically infected people.

All those "maybes" reflect the fact that the evidence so far is sketchy: No one yet knows how—or if—people are actually protected from HIV. But with real-life, real-time AIDS vaccine trials set to begin in 1993, there's little time to waste in finding out.

–Jon Cohen

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