AIDS RESEARCH

Keystone's Blunt Message: 'It's the Virus, Stupid'

Among the many puzzles AIDS presents to medical research, one stands out as particularly critical: The immune system collapses despite the fact that infected people often appear to have only minute amounts of HIV in their blood. That conundrum has bedeviled researchers and even led some iconoclasts, such as virologist Peter Duesberg of the University of California, Berkeley, to suggest HIV isn't the cause of AIDS. But, as several presentations at a recent Keystone Symposium made abundantly clear, appearances can be deceiving. Using creative new techniques for detecting virus that are much more sensitive than previous methods, several scientists have found that there is far more HIV in infected people than was previously thought. Not only do those findings help to put another nail in the coffin of Duesberg's anti-HIV hypothesis, they are also helping to elucidate how the virus undoes its hosts.

Held in Albuquerque, New Mexico, from 29 March to 3 April, the Frontiers in HIV Pathogenesis meeting attracted more than 700 researchers. As in past years, this annual gathering (moved from Keystone, Colorado, after that state passed a law discriminating against homosexuals) gave a state-of-the-art overview of how HIV behaves on the molecular, cellular, and epidemiological levels. David Ho, head of the Aaron Diamond AIDS Research Center and a co-organizer of the conference, plans to make a button that succinctly summarizes the meeting's major theme: "It's the virus, stupid."

That message relates first and foremost to the new findings about high levels of HIV in infected people. AIDS researchers have long thought that the amount of HIV that is present in cells or floating around in the blood remains low until late in the disease. But those assumptions are now being discarded, largely because of remarkably sensitive new uses of the revolutionary technique called polymerase chain reaction (PCR), which amplifies small bits of DNA.

In most previous efforts to measure the amount of HIV in infected people's systems, researchers have relied on standard "quantitative" PCR. Using that technique, researchers measure the amount of HIV in, say, blood cells by extracting the genetic material and adding strands of HIV DNA called "primers." The primers, which typically are radioactively labeled, bind to complementary HIV DNA sequences in the cell; PCR then amplifies the selected sequence many times. The strength of the radioactive signal in the amplified product is then measured.

With this standard assay, the amount of virus detected is directly linked to the efficiency of the amplification process. To the degree that the amplification is less than completely efficient, the amount of virus will be underestimated. Bruce K. Patterson, working in Steven Wolinsky's lab at Northwestern University, has developed a new PCR test that gets around this limitation. Where older techniques found HIV in as few as 1 in 10,000 blood cells, Wolinsky says his group can routinely find HIV genetic material in as many as 1 in 10 blood cells.

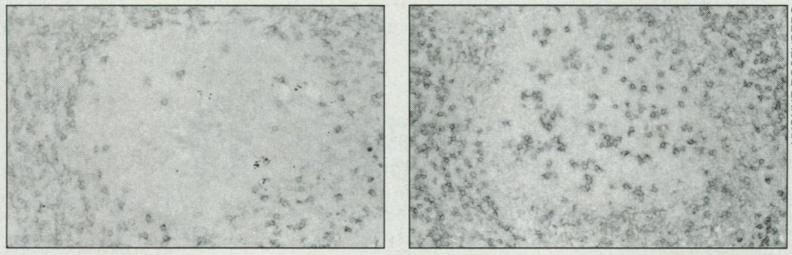
The Wolinsky group's in situ assay requires fixing HIV nucleic acid sequences in the cell, amplifying with PCR, labeling with a fluorescent probe, and then counting the labeled cells. The efficiency of the amplification is less critical than in standard quantitative PCR because the fluorescent probe only needs to find a small amount of PCR product to tag a cell as infected. Using this method, Wolinsky says, his group concluded that "there's more virus than we appreciated. As we improve our technology we improve our ability to find what we're looking for. It's not that it wasn't there before."

Another powerful new viral assay was described by George Shaw and his colleagues at the University of Alabama at Birmingham. As reported in *Science* last month (19 March, p. 1749), the Shaw group, in collaboration with researchers from California's Genelabs Inc., are testing a new technique called "Quantitative Competitive-PCR" (or QC-PCR) that appears to be as much as 60,000 times more sensitive than culture-based plasma viremia assays at detecting HIV in plasma.

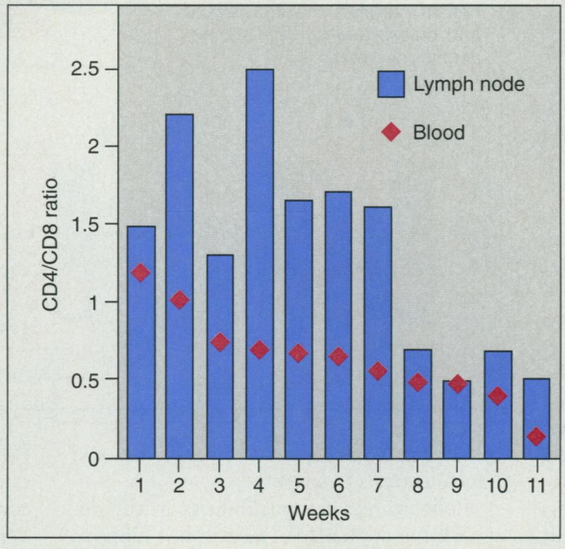
The key feature of QC-PCR is that it simultaneously amplifies HIV nucleic acids and—as a reference—synthetic nucleic acids that are known to create a specific amount of PCR product. Because the amount of amplified HIV is determined by comparing it to the amplified synthetic material, QC-PCR, like Wolinsky's in situ assay, is less vulnerable to the efficiency of amplification than standard quantitative PCR. Perhaps more important, the synthetic material provides an internal control, increasing the accuracy of the notoriously fickle standard quantitative PCR.

The advent of sophisticated new variants of PCR doesn't mean the standard version of this technique is already on the dust heap. On the contrary, standard quantitative PCR is helping researchers understand the critical role of lymph nodes in HIV disease—another focus of the Keystone symposium.

Using standard PCR, Anthony Fauci of the National Institute of Allergy and Infectious Diseases (NIAID) explained how his group compared HIV levels and the amount of viral replication in blood cells and in lymph nodes of people at various stages of HIV infection. Fauci and co-workers found (and published in *Nature* on 25 March) that lymph nodes harbor much more HIV than is found in blood cells in early-stage disease. Furthermore, the lymph node produces virus



YVONNE ROSENBERG



Lymphing along. Data from Yvonne Rosenberg's monkeys *(numbered 1-11)* show that as CD4 levels drop in the blood, they remain high in lymph nodes. Only when the CD4 level drops sharply in lymph tissues does the immune system crash *(animals 8-11)*. Damage to lymph nodes may result from CD8 cells. Left micrograph shows a normal lymph node's "germinal center"; right micrograph shows germinal center invaded by CD8 cells *(dark spots)*.

when little or no active viral production can be detected in blood cells. Fauci's group is trying to explain the disparity between blood and lymph tissue by studying follicular dendritic cells, or FDCs. Lymph nodes are filled with an intricate lattice of FDCs, which filter and trap HIV and other viral particles. As HIV disease progresses and the immune system collapses, the FDC "architecture drops out" of the lymph node and it can no longer trap virus, says Fauci, releasing HIV into the blood.

If the deterioration of the FDC network is linked to the immune system collapse seen in AIDS, it is obviously critical to understand that process. Yvonne Rosenberg, an immunologist at the Henry M. Jackson Foundation Research Laboratory in Rockville, Maryland, is using a primate model to try to explain how the network falls apart. Rosenberg has been investigating lymphoid organs like the gut and spleen from macaque monkeys infected with HIV's simian relative, SIV, and she finds what she calls "a nice correlation" between the disruption of the FDC lattice and the infiltration of white blood cells bearing on their surface immune-system receptors designated CD8—though it's still unclear to her how the CD8 cells are damaging the lattice.

Extending one of the themes of the conference—the relationship between blood and lymph tissue in AIDS—Rosenberg is now questioning the common wisdom about white blood cells bearing the CD4 marker (whose depletion is the hallmark of HIV disease) in blood and lymph. "The assumption has been that if CD4 goes down 50% in the blood, it goes down 50% in the [lymph nodes]," says Rosenberg. "That's just not true."

She found that in her monkeys, the levels of CD4 cells vary in a much more complex pattern. As the ratio of CD4 to CD8 cells declines in the blood, the ratio remains stable in the lymph nodes. Only much later does the fraction of CD4 in the lymph nodes decline—and it is then that immunological trouble really begins. This pattern leads Rosenberg to suspect that initial declines in CD4 cells in the blood are deceptive. CD4s, she thinks, are not disappearing altogether, they're only being sequestered in the lymphoid tissue. If so, this pattern could have some major implications for clinical treatment of HIV disease, since it raises the possibility that to prevent onset of AIDS, researchers should focus on preventing the decline of CD4 levels in the lymph nodes, not in blood.

As many of the presenters at Keystone did, Rosenberg raised as many questions as she answered. Still, a clearer picture is finally emerging of how much HIV there is in the bodies of infected people and where most of it resides. And that means researchers are finally getting a firmer handle on the chain of events that is set in motion by the stealthy entrance of HIV.

–Jon Cohen

MEETING BRIEFS

Chemists Gather in Denver to Get the Big Picture

The Rockies were hidden by clouds, but "Welcome ACS" signs were visible all over the Mile High City from 28 March to 2 April. No wonder the city's boosters were cheering: More than 10,000 scientists, purveyors of laboratory equipment, and other chemophiles had flooded into Denver to take in a vision of chemistry's intellectual scope that was wide enough to compensate for the missing scenic grandeur. In just a tiny sampling of the roughly 4800 presentations, chemistry appears in guises ranging from the most basic molecule making, through cleanup strategies for major environmental challenges, to forensic methods for unraveling a historical mystery.

Mass Spec on the Little Bighorn

June 25, 1876, was the ultimate disaster for General George A. Custer and his men—that much is certain. Beyond that, however, says military archeologist Douglas Scott, there's "more myth than reality" to the generally accepted story of how Custer and more than 200 cavalrymen lost their lives at the hands of Sioux and Cheyenne warriors during the Battle of the Little Bighorn in southern Montana. For a decade Scott, colleagues at the National Park Service's Midwest Archeological Center in Lincoln, Nebraska, and volunteers have been trying to penetrate the cloud of myth. They've now enlisted a new, high-tech ally: accelerator mass spectrometry for analyzing the chemistry of old bones.

Even before chemistry entered the picture, traditional forensic evidence—bullets, cartridges, and other artifacts—had led Scott to some provocative findings. For one, he said, "we found evidence for over 45 types of firearms" among the Sioux and Cheyenne, many far superior to those available to Custer's men. The researchers also argue from the pattern of bullets and spent cartridges on the battleground that, contrary to a common image of Indians swarming over Custer's men, "it looks like there were many well-armed Cheyenne and Sioux" firing from protected positions.

Scott is hoping the bones harbor an equally striking tale. As he told his ACS audience, he and his colleagues are analyzing skeletal remains both from the battleground and from the 10 of Custer's men who were buried as unknown soldiers in the Custer Battlefield National Cemetery. They're searching for subtle chemical differences that should reflect what the men ate in their final weeks.

Using accelerator mass spectrometry, which can detect and precisely measure trace amounts of elements in a sample, Scott's team is measuring ratios of elements such as zinc, strontium, and calcium. Because different foods leave their own elemental signatures in bones, the group's analysis may be able to distinguish bones of officers, who may have eaten diets rich in meat, from those of the enlisted men, who ate more hardtack. Even among enlisted men, differences in mess hall diet might make it possible to tell members of different companies from one another.

BETTMANN ARCHIVE

Custer's last stand. Old bones hint that the battle was less chaotic than historians had imagined.

If so, how bones with different elemental signatures are scattered around the battlefield could help reveal details about the choreography of the battle. Already, says Scott, his team's analysis may have found subtle differences in the elemental ratios between different clusters of remains, suggesting that "we might be seeing [members of] company mess units" who fought and died together. That could mean that the battle was less chaotic than had been thought. Still, the evidence so far won't be enough to dispel all the confusion among historians over Custer's last fight. The "preliminary evidence is tantalizing, but the meaning is not yet clear," Scott says.

The Molecular Bead Game

Imagine threading tiny beads onto a necklace. Now imagine shrinking the necklace by a factor of, oh, 10 million, at which point its