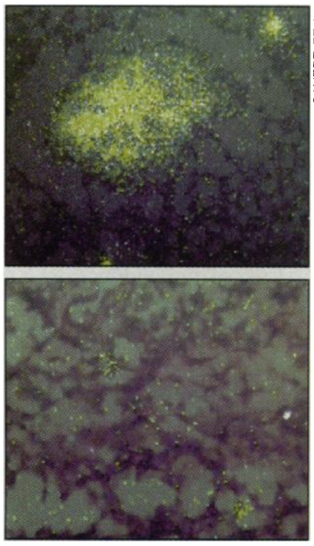
AIDS

Stubborn HIV Reservoirs Vulnerable to New Treatments

In chess, it's called the end game. In football, it's called the longest yard. In the world of AIDS, researchers don't know quite what to call it. But suddenly scientists are tossing around these sorts of metaphors as new treatments have allowed them to beat down HIV RNA in the blood, raising hopes of attacking the virus's other bastions, which so far have remained beyond the reach of anti-HIV drugs. This week, three new reports, one of which appears in this issue of *Science* on page 960, offer insight into how these redoubts finally might be conquered.



CAVERT ET AL.

Dramatic drops. Levels of HIV RNA (yellow) in lymph nodes before and after treatment.

Several recent studies have shown that potent combinations of drugs that attack two critical viral enzymes, reverse transcriptase (RT) and protease, can reduce the amount of HIV RNA in a person's blood to levels too low for the most sensitive assays to detect. But AIDS researchers have cautioned that the virus also hides out in the lymph nodes, which can harbor more virus than can the blood. And HIV is even more impervious to attack when it transforms its genetic material from RNA into DNA, weaves itself into the host's genes, and does not make new viruses—a strategy that allows it to stay under the radar of drugs and the immune system.

The first bastion looks more vulnerable in light of this issue's report by the University of Minnesota's Ashley Haase, Winston Cavert, and their co-workers, who show that a triple-drug combination therapy can dramatically reduce the amount of HIV RNA in lymph nodes. And although no one is reporting any progress in eliminating HIV in its hidden, DNA form, a paper in the 8 May issue of *Nature* sizes up the challenges. Still another report in that same issue explores the rate at which the most persistent HIV-infected cells are eliminated from blood and tissue.

Virologist Steven Wolinsky of Northwestern University says the papers are "critical" to translating scientists' understanding of how HIV causes disease into treatment strategies: "The bottom line is that as long as there's one infected cell left, the job's not done." John Coffin, a retrovirologist at Tufts University in Medford, Massachusetts, adds that these studies "really flesh out some important gaps in previous papers."

One such gap was how triple-drug treatments affect HIV in the lymph nodes. To find out, the Haase group repeatedly biopsied the tonsils (a type of lymph node) of 10 patients who were receiving the RT inhibitors AZT and 3TC, plus the protease inhibitor ritonavir. Over a period of 6 months, the researchers found, the drugs rapidly decreased the levels of HIV RNA inside mononuclear cells, which include two types of white blood cells: scavenger cells known as macrophages, and T lymphocytes that bear a CD4 receptor on their surface (whose decline is a hallmark of AIDS). The drop was not surprising, because mononuclear cells traffic between the lymph nodes and the blood, where the virus already has been shown to be vulnerable to drugs.

More startling was the finding that HIV RNA trapped on the surface of immune cells that form the scaffolding of lymph nodes—follicular dendritic cells (FDCs)—dropped off at roughly the same rate as HIV in mononuclear cells. Based on earlier test-tube studies, says Haase, "we were thinking it would take months, if not years, to clear this virus." Coffin says the result is "quite a novel finding."

Haase cautions that even though the drugs have rid patients' lymph nodes of HIV RNA within a few months, nobody's been cured: "There's clearly a residue of virus still stored on FDCs, and if you stop therapy, there's every indication that you can restart the infection." The Haase group did find one exceptional patient who had no detectable virus in lymph-node mononuclear cells or FDCs after 6 months of treatment. But further study showed that this patient, too, still is infected—with HIV DNA.

The virus's life cycle explains why HIV DNA lingers in the body even after drugs appear to have cleared cells of HIV RNA. When HIV enters a cell, its reverse-transcriptase enzyme changes its RNA genome into DNA—so the virus can integrate its genes into the host cell's DNA. When the cell divides, that viral DNA produces new HIV particles, which once again contain RNA. But often, the HIV-infected host cell remains in a "resting" phase in which it does not divide. The viral DNA, or provirus, in these resting cells is invisible to the immune system and invulnerable to current anti-HIV drugs.

Virologist Winston Cavert, lead author of the *Science* paper, says "the big question" now is how long cells harboring HIV DNA survive, how many such cells exist in an infected person, and how much new virus they can produce. Robert Siliciano of Johns Hopkins University and colleagues addressed these issues by studying HIV DNA levels in resting, CD4-bearing T cells from the blood and lymph nodes of 14 patients, half of whom were receiving treatment. As they report in *Nature*, fewer than 0.05% of the resting CD4s harbored proviral DNA, but many of them, when stimulated to divide, were capable of producing virus. "These cells can survive for months or years," says Siliciano. "They represent the potential barrier for curing the infection."

Because of these and other cells that can coexist with HIV for long periods, the plummeting virus levels seen with the latest therapies may give way to a slower "second-phase decay," say the authors of the second *Nature* paper. David Ho of the Aaron Diamond AIDS Research Center in New York City, Alan Perelson of New Mexico's Los Alamos National Laboratory, and their co-workers reached that conclusion with a computer model, which they used earlier to describe first-phase decay (*Science*, 15 March 1996, p. 1582).

Their model also challenges the dogma that an HIV infection can never be completely cleared from the body. The model takes into account the dynamics in the dance between HIV, cells, and drugs. Untreated, HIV constantly kills the cells it infects, but the virus persists because the body provides it with a steady stream of newly minted cells. Anti-HIV drug treatment prevents the infection of these new cells, and the HIV-infected cells slowly die off. Ho and Perelson's model suggests that the main holdouts are infected macrophages, which are not easily killed by the HIV they produce. By calculating the half-life of these cells, combined with the half-life of infected resting T cells, they estimate that 2.3 to 3.1 years of treatment with a 100% inhibitory anti-HIV regimen might be able to eliminate all remaining virus. Even so, they too conclude with a loud caveat: "Although significant progress has been made in the past year in the treatment of HIV-1 infection, it would be wrong to believe that we are close to a cure for AIDS."

Haase and other researchers contend that mathematical studies of viral kinetics have provided as much information as they ever will about whether it is possible to eradicate the virus. "The only way to answer the question now is to discontinue therapy and measure the virus," says Haase. Ho's group and other labs now have studies under way that should do just that during the next year or so. But Ho stresses that he is not at all certain that the virus will ever be vanquished. "The end game can be as hard as the beginning game or the middle game—and you can lose," says Ho, who formerly was a serious chess player. "I think for HIV, this last bit is the hardest."

–Jon Cohen

ASTRONOMY

Primordial Gas: Fog Not Clouds

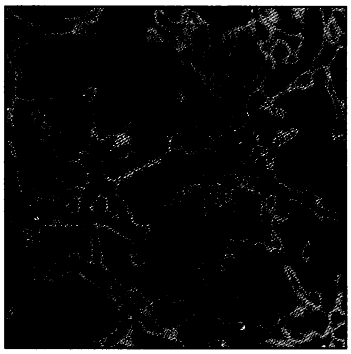
Astronomers lost in an intergalactic forest finally may have found a way out. Two astrophysicists appear to have cleared up the puzzling distribution of tenuous gas in the distant universe, which produces a "forest" of dark lines in the spectra of the bright, remote objects called quasars. By assuming that there is a ubiquitous, undulating fog of hydrogen and helium in the vast reaches of space between galaxies, Arthur Davidsen and HongGuang Bi of Johns Hopkins University not only provide a new explanation for these dark lines, they also account for a key part of the universe's "missing matter."

The new picture, published in the current issue of *Astrophysical Journal*, replaces a model that attributed the forest of lines to discrete gas clouds along the path from Earth to each quasar. Recent computer simulations of how structures took shape in the early universe had cast doubt on the picture, however, and so had the inability of the hypothetical clouds to account for all the hydrogen and other normal matter that cosmologists believe is left over from the big bang. Bi and Davidsen's theory agrees with simulations and provides a home for the missing matter—and it explains the forest of lines to a high degree of accuracy. "[The fact] that observation and theory agree so well means that this is a success—a big success," says David Weinberg, a computational astrophysicist at Ohio State University.

In the early 1970s, astronomers discovered these lines by the hundreds in the ultraviolet region of quasar spectra and dubbed them the Lyman-α forest. Theorists guessed that each line was the shadow of a cloud of hydrogen gas between the quasar and Earth, absorbing light at a specific wavelength. The clouds would be moving away from Earth at different speeds because of the expansion of the universe, and so the Doppler effect would shift each absorption band by a different amount. Hundreds of discrete, spherical hydrogen clouds, each with a different Doppler shift, could explain the whole forest of lines.

But computer models of the universe, which build in assumptions about the amount and types of matter created in the big bang, then trace how the matter coalesces under gravity, couldn't reproduce the clouds. So Davidsen and Bi tried a new approach. They looked at the consequences of an extremely simple mathematical assumption about the intergalactic medium: that the logarithm of its density has a normal, or bell-curve, distribution, meaning that the gas has denser and more tenuous regions but isn't broken into discrete clouds.

A decade ago, the idea would have faced a struggle, explains Davidsen, because the discrete lines in the Lyman-α forest seem to say that the space between the absorbing regions is empty. But astronomers now have reason to think that intervening matter could simply be invisible. The intergalactic medium contains other elements besides hydrogen, among them helium and carbon, and their shadows in the quasar spectra indicate that the medium is highly ionized: Radiation from quasars has stripped away electrons from most of the atoms.



NORMAN ET AL./NCSA

Tendrils of fog. A new picture of the intergalactic medium, as simulated by computer.

As astrophysicist Donald York of the University of Chicago explains, "Ionized hydrogen has no signature; only neutral hydrogen does." Without an electron, a bare hydrogen nucleus can't absorb a photon, so a region of tenuous, ionized hydrogen should leave no trace in the Lyman-α forest. Only in the denser regions would enough neutral atoms remain to create a line.

When Davidsen and Bi used a computer to calculate the precise forest of lines that would be seen in quasar light passing through their continuous medium, they found a close match to the Lyman-α forest. Regions of high density absorb the light at many different wavelengths, much as the discrete clouds did, but they are larger, less dense, and less contrived than the clouds. "There hasn't been a theory which can explain [the lines]," says Mike Norman of the University of Illinois, Urbana-Champaign. "Now that theoretical model is in hand."

Norman, like Weinberg, runs supercomputer simulations of cosmic-structure growth. He too is impressed by the agreement between the Davidsen-Bi picture and the simulation results. The latest computer models generate nothing like the discrete clouds of the earlier picture; instead, they naturally evolve a mass distribution that mimics the Lyman-α forest. Says Norman, "By assuming a distribution for the density, Davidsen and Bi bypassed 500 hours of supercomputer time."

The new picture offers an answer to one of the long-standing problems of astronomy. As David Schramm, an astrophysicist at the University of Chicago, puts it: "Where are the bulk of the baryons?"

According to the big bang theory, the universe should contain several times more baryons—particles of ordinary matter, such as protons and neutrons—than we can see in the stars and galaxies. If gas in the intergalactic medium is in the form of discrete, light-absorbing clouds, there can't be enough of it to account for the baryon deficit. But with highly ionized, and therefore invisible, hydrogen distributed between the dense patches, the diffuse intergalactic medium could account for nearly all the baryonic matter in the early universe—the full amount that had been missing.

Not all astronomers accept the picture, neat as it is. "I don't think that the [baryon-density] value is as high as their model's value," says Lennox Cowie, an astrophysicist at the University of Hawaii. Cowie thinks the new theory and the computer simulations overestimate the amount of ionizing radiation in the early universe and thus the number of invisible baryons. "It's a nice piece of work," he says. "Still, the question's not settled yet."

Others, however, are ready to lay the matter to rest. "We finally feel we understand the Lyman-α forest," says Neal Katz, an astrophysicist at the University of Massachusetts. "Now we can use it as a tool" to study the elusive matter that produces it.

–Charles Seife

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