AIDS RESEARCH

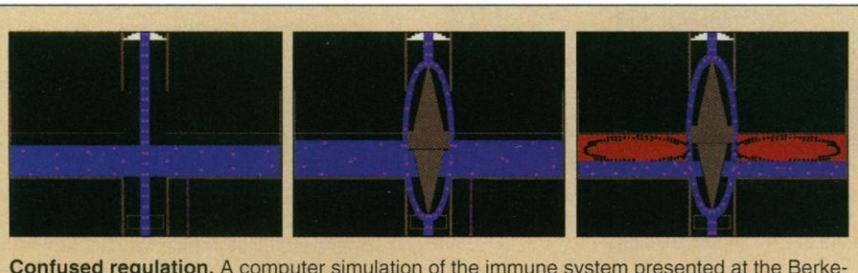
Researchers Air Alternative Views on How HIV Kills Cells

Like a group of radicals from the '60s, two dozen AIDS researchers congregated in Berkeley, California, last month to challenge the establishment, swap copies of their own manifestos, and enjoy the bonhomie of hanging out for 2 days with fellow "alternative" thinkers. The topic wasn't politics, however. Rather, the meeting focused on what has been one of the most puzzling and controversial scientific questions raised by HIV: How does it destroy the immune system and cause AIDS? "We have to subvert the dominant paradigm," said immunologist Michael Ascher of the California Department of Health Services at the opening of the colloquium.*

Ascher was quoting from the philosopher Paul Feyerabend, whose view, said Ascher, was that you don't wait for a paradigm to shift, "you push it over." The paradigm Ascher and his Berkeley colleagues hope to push over is the so-called cytopathic model of HIV pathogenesis. This popular theory holds that HIV cripples the immune system by directly destroying T lymphocytes bearing CD4 receptors, key white blood cells that the body relies on to defeat invading pathogens. It's an appealingly simple theory that test-tube studies back up, and to many researchers, it by and large explains the progressive loss of $CD4^+$ cells in HIV-infected people.

ogy and Respiratory Medicine in Denver. The red flag repeatedly raised by these researchers is that, by their gauges, too few CD4 cells are actually infected by HIV. What is more, they contend, the body can produce new CD4s more quickly than an HIV-infected cell can produce new, infectious HIVs. As immunologist John Krowka of Ascher's group put it, "There are more bodies than bullets." The implication: HIV must somehow be killing uninfected CD4 cells indirectly.

Although the participants in the Berkeley meeting put forward a variety of hypotheses to explain how this occurs, the gathering revealed an esprit de corps rarely seen in the past. The newfound solidarity stems from their misgivings about widespread interpretations of two papers that have been the talk of the AIDS research world since they were published in the 12 January issue of *Nature* (*Science*, 13 January, p. 179). These two independent studies—one led by David Ho, head of the Aaron Diamond AIDS Research Center, and the other by George Shaw of the University of Alabama, Birmingham—analyzed the kinetics of HIV production and its clearance from the body and fluctuations in CD4 counts. Both papers reported that when anti-HIV drugs brought virus production to a grinding halt, CD4 counts skyrocketed.



Confused regulation. A computer simulation of the immune system presented at the Berkeley meeting shows tap-and-drain model *(left)* in which level of reservoir of CD4 cells depends on relative rates of CD4 production and deletion. Researchers at the meeting argued that a regulator adjusts CD4 production and deletion *(center)*. They suggested that during HIV infection, signals from noninfectious virions, depicted by black dots in red foam *(right)*, confuse the regulator, causing the body to produce too few CD4 cells. (Computer simulation by Robert T. Carlson, Carlson Designs; free copies available through bobodoc@well.sf.ca.us)

But to Ascher and the others at the Berkeley gathering he co-organized, the model is too simplistic. "The conclusion that CD4s are killed directly requires a lot of assumptions," said immunologist Terri Finkel of the National Jewish Center for Immunol-

Neither paper overtly argued that CD4s were directly being killed by HIV, but because they both emphasized that billions of virions were being produced each day—which was not so much a new finding as an underappreciated one—that is the message many researchers took home. "It's funny how people read the results differently," says Ho, a virologist. "I just wrote it to say virus is driving the killing. I don't know how, but it is." Yet Ho concedes that he'd put more chips on direct than indirect killing. "Being a virologist, one is very biased to think that way," he says. And, referring to new work from his lab showing even higher levels of HIV replication than in the earlier studies, he adds that "there are more bullets than you think."

The researchers at the Berkeley meeting, several of whom aired their criticisms of Ho's and Shaw's papers in the 18 May issue of *Nature*, were short on compelling evidence that their ideas were correct. But that didn't stop them from vigorously attacking what they perceive as a misguided establishment and from passionately detailing their own visions of how HIV unravels the body's immune tapestry.

Immunologist and Ascher collaborator Haynes Sheppard offered their hypothesis for how HIV undoes the immune system. In a person with a normal immune system, the CD4 population remains constant, making and deleting cells when confronted by an invader. So, somehow, the immune system "counts" CD4s. Sheppard contended that HIV disrupts this homeostasis when its surface protein, gp120, meshes with the CD4 receptor, which signals CD4 cells to become "activated" and mount an immune response. In this model, the excess activation signal calls into action and deletes more troops of CD4s than necessary. "By putting HIV in this system, you essentially give CD4s a false counting system," argued Sheppard.

How, then, are CD4s actually deleted? Sheppard and Ascher think a lead suspect is apoptosis, the process of programmed cell death used to clear unneeded cells. Several speakers at the meeting outlined their own views of apoptotic mechanisms. Finkel mapped out a complex "misactivation" interaction via gp120 binding to the CD4 receptor. In Finkel's model, the binding event renders the CD4 cells anergic, or unable to respond to further stimulation by invaders. More devastating still, when they meet a foreigner, these cells commit suicide.

Finkel also reviewed her recent work, reported in the 2 February issue of *Nature Medicine*, which shows that the apoptosis "kiss of death" primarily takes place when gp120, whether associated or unassociated with HIV, binds to uninfected "bystander" CD4 cells. "HIV may have proteins that inhibit apoptosis in the host cell but trigger it in a bystander," suggested Finkel. One possible agent for inhibiting apoptosis in infected cells is the HIV protein Tat, which she thinks may thwart the gp120-CD4 activation signal and thus protect HIV-infected cells from an early death.

An altogether different explanation for CD4 loss is that it is due to "redistribution" rather than cell killing. CD4s constantly traffic between the bloodstream and remote

* Alternative Models of HIV Pathogenesis, 19–20 July, Berkeley, California.

lymphatic tissue, with the CD4s in the blood representing only about 2% of the total population. Several researchers at the meeting theorized that because HIV progressively destroys the architecture of the lymph nodes, it might also somehow lead the nodes to sequester more and more CD4s than they otherwise would.

Stanford University immunologist Mario Roederer said he believes redistribution is "a very strong possibility." Roederer has been studying how HIV alters the balance between "naive" T cells—ones that have never seen an invader—and "memory" T cells, which have memorized what an invader looks like and committed themselves to attacking it if they see it again. He has found that levels of naive CD4 cells drop much more precipitously in HIV-infected people than do those of memory CD4s. And, curiously, he found that naive CD8 cells—another key immune-system actor—drop in lockstep with naive CD4s, even though memory CD8 cells actually rise during an HIV infection. Because CD8s are not susceptible to HIV infection, Roederer concludes that the synchronized decline in naive CD4s and CD8s cannot be due to direct killing. He favors redistribution, and he also speculates that the loss of naive T cells might be linked to the fact that HIV destroys the thymus, which is where naive T cells are minted.

The University of California, San Francisco's, Jay Levy, a virologist who did not attend the Berkeley meeting, is glad these researchers are encouraging colleagues to re-evaluate the Ho and Shaw papers and the role of direct killing. "[The papers] have value, absolutely," says Levy, who wrote a 106-page tome on HIV pathogenesis in the March 1993 *Microbiological Reviews* and believes indirect killing is key. "But I think they've been over-touted."

Levy hopes the alternative views will lead AIDS clinicians to broaden their thinking beyond anti-HIV drugs. Indeed, the treatment implications stemming from alternative HIV pathogenic mechanisms are many. If false signaling is a critical pathogenic mechanism, for example, then treatments should be aimed primarily at blocking signals. If specific HIV proteins prevent the apoptosis of HIV-infected cells, then those proteins should be targeted. Or if Roederer's hunch is right, perhaps it makes sense to do thymic transplants coupled with therapies that protect the new thymus.

While some of these ideas might seem far-out to AIDS researchers who are banking on anti-HIV drugs, no treatment, to date, has had much success. And unless that bleak reality changes, alternative thinkers will likely keep needling their establishment colleagues and urging them to rethink their basic understanding of the disease.

–Jon Cohen

MEETING BRIEFS

Ecologists Flock to Snowbird For Varied Banquet of Findings

SNOWBIRD, UTAH—About 2500 ecologists converged here from 30 July through 3 August for the largest meeting ever of the Ecological Society of America (ESA). The meeting's theme of the transdisciplinary nature of ecology included talks on such unusual topics as urban ecology and fisheries economics. But there was also plenty of solid ecological fare on tropical forests and evolution.

Forest Fragments Favor Frogs

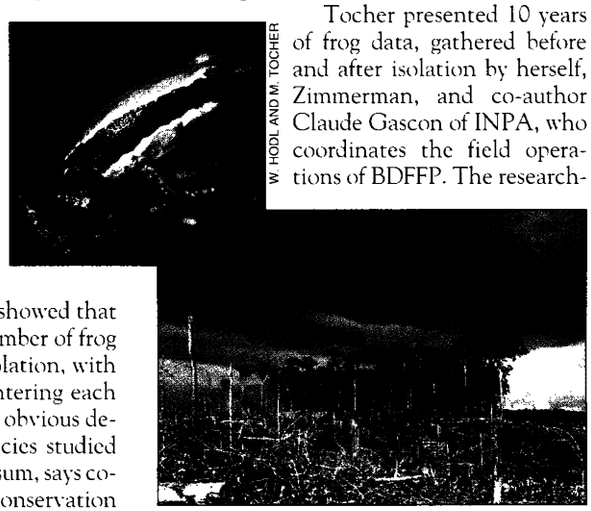
One of the most common landscape alterations in the world today is the conversion of continuous forest into a patchwork of forest fragments surrounded by pasture, farmland, and secondary growth. Ecologists have warned for years that such fragmentation not only wipes out the organisms that lose their habitat, but also harms those trying to survive in the fragments.

So it comes as a surprise to find that in a 10-year experiment in the Brazilian Amazon, frogs—a group thought to be sensitive to disturbance—actually became more diverse after patches of forest were isolated. Results presented at the meeting by Mandy Tocher of the University of Canterbury in Christchurch, New Zealand, showed that in smaller forest patches, the number of frog species roughly doubled after isolation, with an average of 10 new species entering each patch. Frog breeding showed no obvious decline, and only one of four species studied showed a drop in population. In sum, says co-author Barbara Zimmerman of Conservation International, after 7 years of isolation, frogs seemed to do just fine in forest fragments.

The new data haven't turned scientists into advocates of fragmentation. Indeed, the same experiment has shown that in other species, isolation leads to a severe loss of diversity. But this unexpected resilience in a group known to be in worldwide decline may be good news for conservation. The new data bolster the view that what's outside a reserve is crucial to the health of species inside. "Patches are rarely surrounded by completely nonforested areas," says Rob Bierregaard of the University of North Carolina, former field director of the project. "There's secondary growth outside, and it may serve some conservation purposes."

The frog data are part of the Biological Dynamics of Forest Fragments Project (BDFFP) near Manaus, Brazil, begun in 1979 by Tom Lovejoy, now of the Smithsonian Institution, and managed through the auspices of the Smithsonian and Brazil's National Institute for Research in Amazonia (INPA). Lovejoy wanted to find out how large a reserve must be to save the species in a given area, so he and colleagues marked off forest patches ranging in size from 1 to 100 hectares. Ranchers and farmers cleared surrounding land and isolated the patches, although tall secondary growth now adjoins some fragments.

Tocher presented 10 years of frog data, gathered before and after isolation by herself, Zimmerman, and co-author Claude Gascon of INPA, who coordinates the field operations of BDFFP. The researchers surveyed frogs by sight and sound (frog mating calls are distinctive), and also surveyed tadpoles in breeding ponds.



W. HODL AND M. TOCHER

ROB BIERREGAARD / WWF

Tree-mendous diversity. Fragmentation of this Amazonian forest gave frogs a surprising boost.

Because Amazonian frogs typically have strict physiological and breeding requirements, researchers predicted lower frog diversity, abundance, and breeding success, especially in small fragments. But they were wrong. Although larger patches did have more diversity than smaller ones, all fragments had more frog species after isolation than before.

This is all the more surprising given that BDFFP and other experiments have already shown that isolation is usually bad for diversity. At the symposium, Gascon presented published and unpublished summary data from various BDFFP researchers showing a diversity decline after isolation in birds,