AIDS Mood Upbeat—For a Change

The intersection of basic research and clinical findings seems to have provided some new and promising leads in the struggle against HIV

Last year, Harvard's Bernard Fields, a respected virologist but an outsider to AIDS research, rocked that field when he argued in a *Nature* commentary that too much emphasis was being given to treatments and vaccines and not enough to basic research. His editorial touched a nerve because it crystallized what many AIDS researchers were themselves beginning to admit: They still lacked a basic understanding of how HIV works, and as a result, have yet to discover a drug or vaccine that can outwit the virus. Fields' editorial prompted an invitation for him to kick off an AIDS conference held in Washington, D.C., 2 weeks ago.* But because of Fields's bout with another intractable disease, pancreatic cancer, he could not attend. He wasn't absent in spirit, though, as many speakers at the meeting provided ample evidence that the back-to-basics approach can pay off clinically.

Indeed, Fields' replacements to open the meeting reinforced the point by describing their eye-opening basic research findings about how HIV cripples the immune system. Those clinicians, George Shaw of the University of Alabama, Birmingham, and David Ho of the Aaron Diamond AIDS Research Center, received much attention last month when they published studies revealing that millions of HIVs are produced and removed from the blood of infected people every day (*Science*, 13 January, p. 179). At the D.C. meeting, two other groups independently reported similar data, increasing the excitement over the work.

This strong intersection of fundamental and clinical questions set the tone for the entire 5-day gathering—as did a notable absence of gloom. Recently, many AIDS meetings have been downbeat, with the hottest topic being the unraveling of apparently exciting findings that had come out in the months prior to the meeting. But the 2300 scientists who attended the 2nd National Conference on Human Retroviruses and Related Infections seemed buoyed by reports of steady, incremental progress from the lab bench to the clinic.

One of the most encouraging findings reported was more evidence that the cause of Kaposi's sarcoma (KS), an AIDS-related malignancy that leads to purple skin splotches and many deaths, indeed has been found. Among other intriguing presentations at the meeting: promising data about two AIDS drugs used in combination—AZT and the experimental preparation 3TC; new light on AZT drug resistance from data showing that when HIV becomes resistant to 3TC, it may undo the established resistance to AZT; and important clues regarding the function of the HIV protein Nef, which many researchers believe is linked to HIV's destructive force.

Immunologist Robert Schooley of the University of Colorado, chair of the scientific committee that organized the meeting, was delighted by the resurgent optimism. "People were more upbeat than I've observed in several years," says Schooley. "We're beginning to see much more consistent progress over a broader array of fronts."

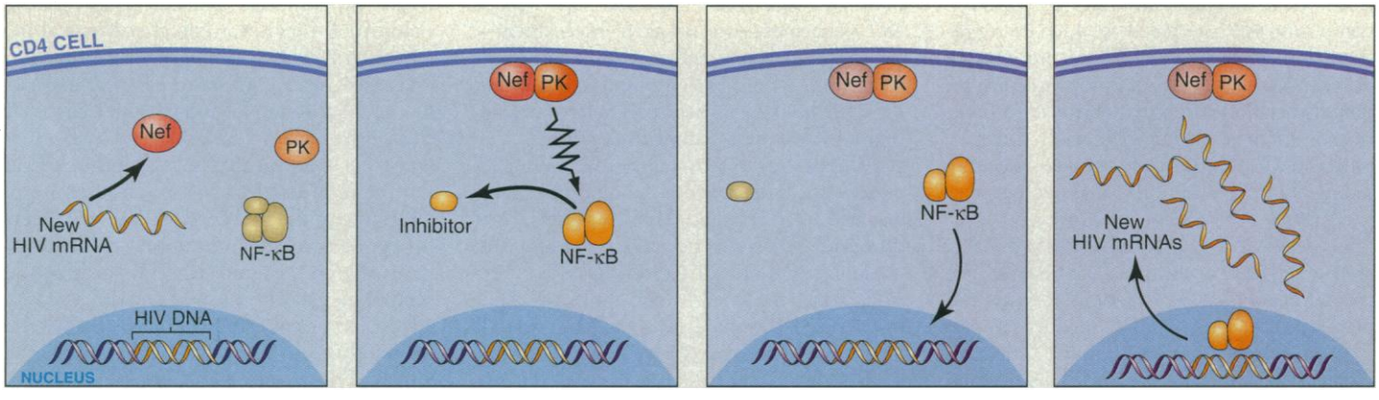
Among the more dramatic examples of progress was the strengthening evidence linking a new virus to KS. Last December, Patrick Moore, Yuan Chang, and their collaborators stunned AIDS researchers when they reported in *Science* that they had found a prime suspect. Moore and Chang, Columbia University researchers who were virtual unknowns in the AIDS research world, showed how they had isolated fragments of what appeared to be a new herpesvirus from KS lesions in people with AIDS; the sequences were not found in samples from healthy controls.

At the D.C. conference, Moore reported that although they have yet to observe the virus in an electron micrograph, they have transmitted it to a cell line and identified two dozen of its genes. They also know that its DNA includes 270 kilobases (270,000 nucleotide base pairs), making it the largest known herpesvirus.

In spite of this accumulating evidence, Moore and Chang were reluctant to declare that they have found the cause of KS, but others at the meeting were less restrained. Steven Miles, a KS researcher from the University of California, Los Angeles, who initially had serious reservations about the putative new virus, enthusiastically embraced the new findings. "I'm convinced that it is a herpesvirus, and it is very definitely the cause of Kaposi's sarcoma," said Miles, whose lab has replicated Moore and Chang's initial work.

The Columbia researchers also revealed data at the meeting answering a critical question that wasn't addressed in their initial report. Although AIDS has made KS famous, a mild form of the malignancy has long been

*2nd National Conference on Human Retroviruses and Related Infections, 29 January–2 February, sponsored by the American Society of Microbiology, Washington, D.C.



SOURCE: S. LANG AND R. DESROSIERS/NERPC; M. PETERLIN/UCSF

A real turn-on. The HIV protein Nef associates at the cell membrane with a protein kinase (PK); the PK removes an inhibitor from NF-κB; the de-inhibited NF-κB goes to the cell nucleus, where it turns on production of more HIV RNA, driving viral replication upward.

studied in people not infected with HIV. If the new herpesvirus indeed causes KS, its genetic sequences should be found in HIV-negative people with the disease. And that is just what Moore and Chang reported at the meeting. They analyzed six samples from people with "classical KS"—a disease that usually afflicts elderly Mediterranean men—four from HIV-negative gay men with KS and 10 from Ugandans with "endemic African KS." Without exception, the samples were positive for the herpesvirus DNA sequences. The researchers are now trying to develop a blood test for antibodies to the virus, which could swiftly assess whether their findings hold true for the population at large.

Resensitivity training?

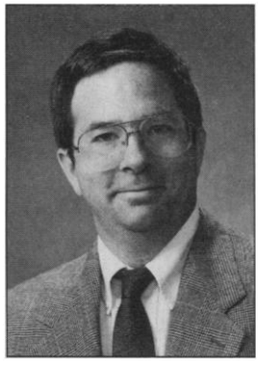
Aside from the KS data, one of the most positive aspects of the meeting was the presentation of data confirming Ho and Shaw's results, which many AIDS researchers believe constitute significant headway into understanding the battle between HIV and the immune system. Their work indicates that while the level of HIV in the blood remains constant over, say, a month, in fact massive amounts of the virus are being pumped into and removed from the blood every few days. A study from Douglas Richman's lab at the University of California, San Diego (UCSD), presented at the D.C. meeting backed the Ho and Shaw data, as did another one from Clive Loveday from the University College London Medical School.

For researchers developing anti-HIV drugs, these studies send a grave message: This virus replicates at a breakneck speed and therefore may be an even tougher foe than has been thought so far. Still, treatment strategies are inching forward, as the new data from studies of AZT combined with 3TC show. Addressing a standing-room-only crowd, researchers conducting two multicenter, North American trials reported that combining AZT with 3TC yielded better results than either alone. These preliminary data confirm results from European trials reported last fall, and they may bolster the reputation of AZT, bruised in 1993 when Europe's powerful Concorde study showed the drug did not help healthy, HIV-infected people.

A major note of caution, however, is that none of the AZT/3TC studies has yet evaluated whether the combination reduces the incidence of AIDS-related infections and prolongs the lives of HIV-infected people. Instead, studies to date have analyzed changes in the amount of HIV in treated people and changes in the immune system—"surrogate markers" that presumably are signposts of whether a treatment is working. "It appears to be a promising therapy," said Joseph Eron Jr. of the University of North Carolina at Chapel Hill, who presented surrogate marker data from one such trial.

This trial involved 364 HIV-infected patients who received either the AZT/3TC combination, at different dosages, or one of the two drugs alone. All the participants were "AZT naive," meaning they had less than 4 weeks's prior treatment with the drug. At the start of the trial, they had between 200 and 500 CD4 cells per cubic millimeter of blood. (CD4s, critical white blood cells, normally range from 600 to 1200 per cubic millimeter.)

After 24 weeks, patients receiving a high dose of 3TC along with AZT saw their mean CD4 count jump by 58 cells above baseline and the total level of HIV genetic material in their plasma—a measure of "viral load"—drop by 90%. In contrast, people taking 3TC alone experienced a mean increase of only 15 CD4s at 24 weeks and a 67% reduction in viral load; AZT alone also had less impressive effects than the combination. Early data suggest that these trends are still apparent, although not as strong, at 1 year. And although these data were too preliminary to assess whether AZT/3TC is more powerful than the already approved combination of AZT and ddC, AIDS clinicians still took heart. "It's encouraging," says UCSD's Richman, who says he is impressed both by the magnitude of AZT/3TC's effects on surrogate markers and the positive trends seen a full year into the study.



Sees progress. Colorado's Robert Schooley.

Reflecting the generally upbeat frame of mind, researchers at the meeting were even putting a positive spin on the uncanny ability HIV has to develop resistance to drugs quickly. Brendan Larder and his colleagues at the U.K.'s Wellcome Research Labs, a sister company to AZT's manufacturer, analyzed the amino acids of the HIV reverse transcriptase (RT) enzymes—the target of both drugs—isolated from 50 patients in a European AZT/3TC trial. These patients had been taking AZT before the trial's start and had many RT mutants that are resistant to the drug.

When they began taking AZT/3TC, a curious thing happened: As mutants resistant to 3TC cropped up, the viral loads dropped. Alhough he can't explain the mechanism, Larder theorizes that the mutation that made RT resistant to 3TC resensitized the same RTs to AZT, a trick that's been noticed with other combinations. "Maybe 3TC resistance is a good thing rather than a bad thing," concluded Richard D'Aquila of Massachusetts General Hospital in his talk about the clinical significance of resistance.

Back to basics

Another area that for a change seems to be generating more light than heat is the function of the HIV regulatory protein Nef. Several investigators contend that Nef paves the way for the virus to cause disease, and new data to support this idea were presented by Ronald Desrosiers of the New England Regional Primate Center. Desrosiers and coworkers focused on the Nef protein of an exceptionally lethal strain of SIV—the simian AIDS virus—called pbj14, which kills pigtail monkeys in 2 weeks. Desrosiers's new work suggests Nef may be a component of the virus's quick killing capacity.

Desrosiers and colleagues began by comparing pbj14's Nef to the one found in a less lethal strain called mac239. They found two critical amino acid differences between the two Nefs. They then engineered a mac239 that expressed a Nef with the two pbj14 amino acid changes. In test tube studies, this modified mac239 grew at much higher rates than before. "The virus grows great guns," said Desrosiers. They next put the modified mac239 into 12 monkeys. Although the animals did not die rapidly, they did develop the severe symptoms characteristic of pbj14 quickly.

The intriguing aspect of this finding is that the two amino acid changes occur in a region of Nef that controls binding to protein kinases, enzymes that start a cascade of signals from the cytoplasm of cells to the nucleus. In HIV-infected cells, one theory holds, the protein kinase might signal a molecule called NF-κB to scuttle one of its components, the inhibitor I-κB. NF-κB can then migrate into the nucleus and trigger the transcription of HIV, revving the cellular machine that cranks out new virions. Desrosiers suspects that the Nefs in pbj14 and mac239 both have similar functions—a two amino acid change, he reasons, should not alter the protein that much—but that pbj14's Nef is simply more efficient.

The connection to the protein kinases backs up findings presented by Matija Peterlin of the Howard Hughes Medical Institute and the University of California, San Francisco, whose lab last year revealed the first link between Nef and protein kinases. "I think [the pbj14 data are] very intriguing, and it fits very nicely," says Peterlin. David Baltimore of the Massachusetts Institute of Technology also presented data at the meeting supporting the protein kinase–Nef link.

In the end, the meeting was exactly the kind of merger of basic and clinical research advocated by Bernard Fields. Sadly, he wasn't there to see it: On 1 February, the fourth of the meeting's 5 days, Fields died at age 56 of his pancreatic cancer.

–Jon Cohen