Exploiting the HIV-Chemokine Nexus

Researchers now have an understanding of the intricate mechanism by which the AIDS virus enters cells, and they are racing to turn this understanding into new therapies

When authors report basic biomedical research results, they traditionally end their papers with a few words about how their abstruse findings may one day benefit human health. This discussion often seems to be an afterthought, designed more to please funders than to offer real possibilities of new treatments. And there's usually good reason for reticence: The gap between basic and applied research is generally vast, and talk of bridging it often is highly premature. Yet, a basic research revelation can sometimes spin a field on its head and immediately open up new possibilities for important applications. Just such a development is now energizing the world of AIDS research.

It began just 14 months ago, with a paper that ended on a laconic note: "[These results] may open new perspectives for the development of effective therapeutic approaches to AIDS." The paper uncovered a link between HIV and the then-obscure immune system messengers called chemokines. Since then, a surge of results has shown just how intimate this relationship is: HIV slips into cells by commandeering receptors on their surfaces that normally bind to chemokines. And these findings have answered one of the big mysteries of AIDS research: how HIV infects cells.

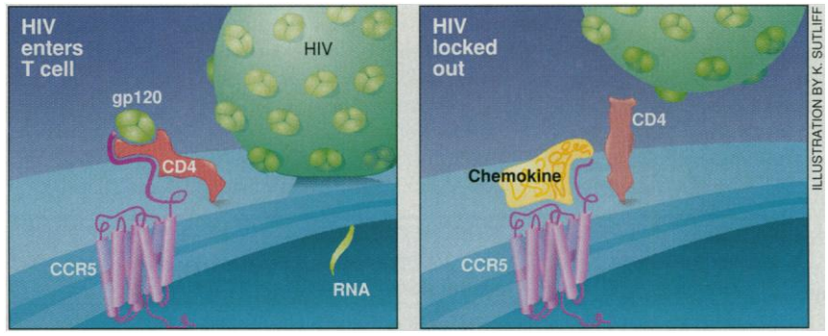
A pack of academic teams, biotechnology companies, and big pharmaceutical houses are now racing to develop treatments that exploit this HIV/chemokine nexus. Researchers are also aggressively investigating whether chemokines can help explain why some AIDS vaccines work in primates and others do not. And intense efforts are under way to use the chemokine discoveries to genetically engineer a small animal to make it susceptible to HIV infection—research that could lead to a long-sought model for studying the disease.

"The field is moving so rapidly it's painful to keep up," says Anthony Fauci, head of the National Institute of Allergy and Infectious Diseases (NIAID). But he notes that efforts to apply all this new knowledge are running into plenty of complications. "Every week, you see the complexity of the receptor [story] get more intense," says Fauci. Adds the University of Pennsylvania's Robert Doms, whose lab has helped trace the connection between chemokine receptors and HIV, "The whole surface is bubbling here. We'll see what erupts."

Entry criteria

Like most AIDS researchers, Robert Gallo knew next to nothing about chemokines in the fall of 1995. But he got a crash course in these molecules when Paolo Lusso, Fiorenza Cocchi, and other researchers in his lab, then at the National Cancer Institute, first discovered that certain chemokines powerfully abated the growth of HIV in lab cultures.

Chemokines, which are produced by a wide variety of cell types, are the paging system of the inflammatory process, recruiting white blood cells to injured or ailing tissues. As Gallo, Lusso, and their colleagues detailed in a seminal 15 December 1995 *Science* paper, three chemokines known as RANTES, MIP-1α, and MIP-1β have an uncanny knack for inhibiting strains of HIV recently isolated from patients. Oddly, though, they found that these chemokines had little effect on HIV that had been grown in immortalized lab cultures of white blood cells called T lymphocytes.



Blocking the door. CD4 receptor binds to gp120 on HIV's surface, forming a complex that binds to CCR5 *(left)*. When a chemokine—or a drug—occupies CCR5, HIV is shut out.

At the time, the Gallo group didn't know how the chemokines kept HIV in check or why they inhibited only "primary" HIV isolates. A huge piece of the puzzle fell into place the following spring, when Edward Berger and colleagues at NIAID answered the question of how HIV enters cells.

Researchers have long known that HIV uses a T-lymphocyte receptor called CD4 to infect cells, but it has also been clear for more than a decade that the virus needs another factor—possibly a second receptor—to do its dirty work. Berger and co-workers identified a receptor now known as CXCR4 as that missing factor. And they correctly surmised that it belonged to the chemokine family based on its amino acid sequence. Yet, Berger's results added a new twist: CXCR4 seemed to provide a point of entry for HIVs grown in cell lines, but not primary HIVs.

The direct tie-in to the Gallo lab's work came in late June 1996, when five labs, including Berger's, reported in back-to-back *Science*, *Nature*, and *Cell* papers that primary HIVs use a different chemokine receptor, now dubbed CCR5. This receptor normally binds RANTES, MIP-1α, and MIP-1β, suggesting that these chemokines inhibit HIV by blocking some of its entrances to the cell.

The crucial role that CCR5 plays in early infection was made crystal clear a couple of months later. That August, independent research teams reported in *Nature* and *Cell* that several people who had repeatedly had sex with infected partners but remained uninfected themselves had a mutation in the gene that codes for CCR5. Several studies since then have confirmed that mutant CCR5s make people highly resistant to HIV infection. "That's a staggering observation," says primate researcher James Stott from the U.K.'s National Institute for Biological Standards & Control. (This defect may not completely protect people, though: The March issue of *Nature Medicine* has a letter from Robyn Biti and colleagues at Westmead Hospital in Australia about an infected man whose cells have the mutant CCR5s.)

A great deal of work now has connected the dots between different strains of HIV and the chemokine receptors they rely on. HIVs that cause the initial infection predominantly use CCR5, while—for reasons that are still being keenly debated—the HIVs that predominate in the final stages of disease resemble the viruses grown in T-cell lines and bind to CXCR4. Virologist Robin Weiss at the Chester Beatty Laboratories in London cautions, however, that this picture probably will prove simplistic. "It's not as though the

work over the past 6 months is going to be overturned, but things are sure to get more complicated," says Weiss. "Watch this space."

Many inhibitions

Provisional as these basic research findings are, researchers are tripping over each other to translate them into practical applications, such as vaccines and drugs to treat people who are already infected. Many believe that treatments are the more promising avenue. "That's the one that's most likely to come to fruition the fastest," says virologist Joseph Sodroski, a veteran HIV-entry investigator at Boston's Dana-Farber Cancer Institute. It's also a critical area: Despite recent progress with combinations of drugs that cripple HIV enzymes (*Science*, 20 December 1996, p. 1988), the treatments don't work for everyone, and, as time goes on, drug-resistant strains of the virus are sure to become an ever greater problem.

Big pharmaceutical companies are going after this challenge with great gusto (see table), largely because they're on familiar turf: They have already developed enormously profitable drugs—including leading ulcer medications—that target "7-transmembrane" receptors, the family to which chemokine receptors belong. "The biggest drugs in the world are inhibitors of 7-transmembrane spanners," says Thomas Schall, a pioneering chemokine investigator who works at DNAX Research Institute in Palo Alto, California, a division of the drugmaker Schering-Plough.

"As soon as these [HIV] coreceptors were described in the literature, given our expertise in 7-transmembrane receptors, we put together a screening strategy [for drugs to block them]," says Lawrence Boone, a virologist at Glaxo Wellcome in Research Triangle Park, North Carolina. What's more, Glaxo and several of its competitors already had programs under way looking specifically for chemokine-receptor inhibitors to treat such inflammatory diseases as asthma, rheumatoid arthritis, and psoriasis. Harvard University inflammatory disease specialist Craig Gerard, who now collaborates with Sodroski on HIV, says that companies also are aware that one such drug could reap enormous profits if it proved effective against both an inflammatory disease and AIDS, which he says is a "distinct possibility."

Drug developers are strongly encouraged by the fact that people with CCR5 mutants don't have obvious health problems, which suggests that blocking the receptor will not directly cause harm. "Drug companies would ordinarily spend a lot of money" addressing the very question that nature has already answered, says Gerard. Indeed, molecular virologist Richard Colonno of Bristol-Myers Squibb in Wallingford, Connecticut, says the finding that people with defective CCR5s appear to be both highly resistant to HIV and healthy has had a big impact on his company's decision to enter this race.

Like most of its competitors, Bristol-Myers is looking for a small molecule "antagonist" that blocks CCR5 and ideally can be given as a pill. The search typically begins with assays—which often owe much to other 7-transmembrane work—that can screen hundreds of thousands of compounds to see whether they can bind the receptor. Those that show promise are then put through a more complicated battery of tests to determine whether they can prevent HIV from infecting cells. Drugs that make it past that stage are tested in animals to analyze metabolism rates and toxicities. "Most folks are in the same phase: They've gone through the primary screen, and they're looking to see if they can inhibit HIV," says Schall, who notes that his company is looking for drugs against CXCR4 as well.

Some companies are trying variations on this theme. LeukoSite, a Cambridge, Massachusetts, biotech, has teamed up with Warner-Lambert's Parke-Davis to look for a small-molecule CCR5 inhibitor, but it is also searching for monoclonal antibodies to CCR5. The company already has identified eight antibodies that bind to CCR5 and block it in test tube studies. LeukoSite immunologist Charles Mackay acknowledges that antibodies have several disadvantages compared to small molecules: They have to be injected, they are expensive, and they can only be used for a few months before the immune system mounts a response against them. Still, he says small molecules typically have more toxicities than natural molecules like antibodies.

Boone says his company is taking a different cue from nature: It is looking for an "agonist" that, by mimicking natural chemokines, would hit HIV with a double whammy. Not only would it block CCR5, but the binding process would trigger the receptor to send out a signal to tell the cell to hunker down and express fewer of its CCR5s—the same signal normally generated by a chemokine. Indeed, Gallo, who now heads the Institute of Human Virology at the University of Maryland, Baltimore, thinks that chemokines themselves may be promising drug candidates. Although he notes that many researchers have warned that giving chemokines could lead to serious toxicities, he says, "We don't have any toxicities yet, and we've gone up to pretty high doses [in animal tests]."

Two companies, wary that inappropriate signaling by natural chemokines could have dire consequences, are developing modified versions of chemokines that bind CCR5 but do not act as agonists. At Glaxo Wellcome in Geneva, Timothy Wells and co-workers are working on variants of RANTES that bind CCR5. "My best guess is the sheer amount of material you have to give is still an issue," says Wells. The other company, British Biotech, already is doing human testing of a MIP-1α variant called BB-10010 in cancer and HIV studies. "People expected it would be inflammatory, but it's just not," says Lloyd Czaplewski, who is heading the project.

Researchers caution, however, that even if some of these potential treatments lower HIV levels and are well tolerated, they could be tripped up by the same factor that has sent

SOME THERAPEUTIC APPROACHES

Developer	Collaborators	Approach
British Biotech	Ian Weller, Ian Williams, U. College London Jean McKeating, U. Reading	Modified MIP-1α
Bristol-Myers Squibb		Small molecules
Chiron		Declines to reveal
CombiChem	Edward Berger, NIAID	Small molecules
Glaxo Wellcome Research Triangle Park	Jim Hoxie, U. Penn Mark Marsh, Imperial College	Small-molecule antagonists, agonists
Glaxo Wellcome Geneva	Paul Clapham, Chester Beatty Labs	Modified chemokines
Immusol	Flossie Wong-Staal, UCSD	Ribozymes
Institute of Human Virology	Chris Owman, Wallenberg Labs Chris Tan, Inst. of Cellular and Mol. Biol., Singapore	Chemokines
LeukoSite	Craig Gerard, Harvard and ADARC	Antibodies
Merck		Small molecules
Pfizer		Small molecules
Progenics	ADARC	Small molecules, modified chemokines, antibodies
Schering-Plough	DNAX	Small molecules
Warner-Lambert/ Parke-Davis	LeukoSite	Small molecules

HIV Experts vs. Sequencers in Patent Race

HIV researchers have electrified the field for the past year with a string of discoveries that revealed in detail how the AIDS virus grapples onto and enters certain human cells. At least five scientific teams zeroed in on one molecule in particular—the CCR5 receptor on immune system cells—and found that it acts like a key, opening the cell to HIV infection. If the receptor is absent or altered, the invader has trouble getting in.

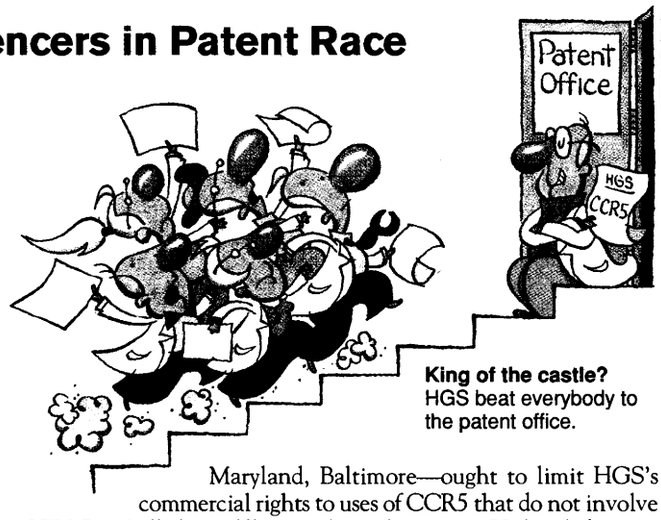
This is high-impact science, with high commercial stakes as well: Some observers predict that the CCR5 discovery will lead to new drugs designed to block HIV infections (see main text). It should come as no surprise, therefore, that half-a-dozen groups are vying for priority on CCR5, and many are filing patents. But these competitors may themselves be surprised to learn that a company that was not directly involved in these HIV studies—Human Genome Sciences (HGS) of Rockville, Maryland—appears to have beaten everyone to the patent office.

William Haseltine, HGS's chair, confirms that HGS applied for a patent on the DNA sequence coding for the CCR5 receptor back in June 1995, long before the recent scientific reports were published. HGS's early claim points up an issue that's likely to be more and more vexing to DNA patent seekers. Since the early 1990s, companies doing large-scale DNA sequencing have been filing claims on thousands of genes and gene fragments, often without knowing exactly what the DNA codes for. HGS has been among the most aggressive in this game, and CCR5 may be one of the big fish it has snagged.

HGS's chief patent counsel, Robert Benson, declines to talk about the company's pending application at the U.S. Patent and Trademark Office. (The U.S. review process is confidential.) But Benson did provide a copy of HGS's international patent filing (WO 96/39437). It was released in December, in compliance with an international treaty requiring that such applications be published 18 months after submission. An expert in this field, Edward Berger of the National Institutes of Health (NIH), after hearing the sequence, confirmed that it is the same CCR5 sequence he and others have reported.

HGS said in its patent application that it had found a gene for something it "putatively had identified as a chemokine receptor." HGS asked for rights to variations on the sequence and claimed a list of wide-ranging applications, from uses in gene therapy to drug manufacturing to disease monitoring. But HGS did not guess at CCR5's role in HIV infection. In fact, it didn't even mention HIV.

This omission, according to HIV experts like Robert Gallo—director of the Institute of Human Virology at the University of Maryland, Baltimore—ought to limit HGS's commercial rights to uses of CCR5 that do not involve HIV. But Gallo himself has a stake in this matter. He headed a team of NIH scientists that discovered in 1995 that chemokines play a key role in HIV infection. After the report was published, other researchers zeroed in on the chemokine receptors. In 1996, they identified two of them—CXCR4 and CCR5 and their variants—as key to HIV infection. Many of these teams have now filed for patents on these discoveries, including NIH, Gallo's new institute, and a group led by Marc Parmentier at the Free University of Brussels—the first to make the CCR5 sequence public last spring.



King of the castle? HGS beat everybody to the patent office.

But the quality of this scientific research may have little bearing on the authors' commercial rights. As HGS's Benson says: "Scientific credit is one thing; patent law is another." HGS's outside attorney, Jorge Goldstein of the Washington, D.C., firm of Sterne, Kessler, Goldstein & Fox, explains that whoever is first to patent a DNA sequence—for any use—can lock up subsequent uses. A patent of this type is called a "composition of matter patent," and it prevents anyone from using the DNA sequence without the patentee's permission. If a later inventor patents a new use, Goldstein says, it may create a stalemate in which neither patent-holder prevails. The common solution is to negotiate a cross-licensing agreement and share royalties.

It remains to be seen whether HGS will actually win a patent on the CCR5 sequence. If it does, several other teams of biologists will be disappointed. But Goldstein says that "for 100 years, chemists have known that getting a [composition of matter] patent on a compound is the key." And he adds that it's time for biologists to wake up and "discover the patent system in all its glory." **–Eliot Marshall**

many anti-HIV drugs to an early grave: resistance. Indeed, in theory, HIV mutants might resist drugs that block, say, one part of CCR5 but not another. Even worse, a CCR5 drug could encourage the growth of a virus that prefers CXCR4; while it's far from clear-cut, HIV strains that use CXCR4 may cause disease more quickly.

Biochemist John Moore of the Aaron Diamond AIDS Research Center (ADARC) in New York City worries that companies are going to exaggerate their early findings in HIV trials with chemokine-receptor blockers. "I think there's going to be a lot of hot air and smoke," says Moore. "Exploitation clinically? Come back in a couple of years."

Vaccine dreams

The wait for a payoff likely will be even longer when it comes to vaccines. But some researchers believe the time line can be shortened if the new chemokine work helps answer a big mystery: Why do some AIDS vaccines protect animals from "challenges" with infectious doses of the AIDS virus?

AIDS vaccines have been tested most extensively in monkeys, which develop an AIDS-like disease when they are infected by a close kin of HIV called SIV. Although several vaccines have protected monkeys from SIV infection, no one has yet convincingly elucidated the mechanism behind that protection. Some studies suggest that the protection correlates with vaccine-induced anti-SIV antibodies, which "neutralize" the virus before it infects cells. Other experiments point to cytotoxic T lymphocytes (CTLs), which selectively kill already-infected cells, as a key correlate of protection. But in yet other studies, neither CTLs nor antibodies explain much of anything. Now, primate researchers are looking for a correlation in chemokine levels—and they are finding potentially promising leads.

The first such study appeared in last July's *Nature Medicine*. Thomas Lehner of United Medical & Dental Schools of Guy's Hospital in London reported that high RANTES, MIP-1β, and possibly MIP-1α

levels correlated with the complete or partial protection of seven monkeys. In 13 unvaccinated control animals that easily became infected by the challenge virus, chemokine levels were much lower. This suggests that the vaccine, by some unknown mechanism, stimulated the immune system to produce higher levels of these chemokines, which in turn blocked receptors needed by SIV and prevented infection. "I do not believe any single candidate is the correlate of protection," says Lehner. "But I think [these chemokines] are at least as good a candidate as any of the others."

Lehner currently is conducting experiments to follow up on this work but, for competitive reasons, declines to describe them publicly. "I'm amazed at the speed at which this is moving, and there's a total silence about what people are doing," he says. Jonathan Heeney, an AIDS researcher at the Biomedical Primate Research Centre in Ryswick, the Netherlands, is tight-lipped, too, but says he recently completed a study that looked at chemokines and the protection offered by an AIDS vaccine in monkeys. "We've got a hint that there's something interesting going on there," says Heeney.

Marc Girard of the Pasteur Institute in Paris says he also has intriguing preliminary data from studies of chimpanzees given HIV vaccines. Girard challenged four vaccinated animals and one control chimp with HIV, which readily infects these primates but doesn't usually cause disease. Three vaccinated animals were protected, and all had higher levels of RANTES, MIP-1α, and MIP-1β than the one that became infected. (Unfortunately, the control animal did not become infected, confusing the results, but it, too, had elevated levels of these chemokines.) "We had very good correlation between high level of secretion of chemokines and protection," says Girard. But he doubts that chemokines are the sole explanation and says he needs to repeat the experiment.

Gallo is convinced that chemokines play a large role in protection, which he is attempting to prove by directly injecting them into monkeys and then challenging them. "I think chemokines and CTLs are going to be the answer for vaccines," says Gallo. If indeed his challenge experiments succeed, the next hurdle—and it too is a high one—will be to design a safe vaccine that can teach the immune system to boost production of these chemokines should it ever meet HIV.

New models

The third quest invigorated by the chemokine discoveries is the search for an animal that can develop AIDS. Experiments with monkeys and chimps have provided critical data for AIDS drug and vaccine developers, and for researchers studying disease progression. But these primates are expensive and, except for a few cases in chimps, they do not actually get sick from HIV. So, several groups now are trying to use the new chemokine-receptor advances to genetically engineer a small animal that would provide a more practical model. The aim is to create animals that sprout CD4s and the various HIV-related chemokine receptors on their cells. This effort, too, is far from a shoo-in.

Most researchers working in this area have focused on genetically engineering HIV-infectable mice. Although several groups are believed to have succeeded in getting these receptors expressed, that's just the first step. "For those who think it's just sticking these genes in and making a mouse that's infectable, I think they'll be disappointed," says Dan Littman of New York University's Skirball Institute, whose lab is a leader in this field.

One major problem is that even if HIV can be induced to enter a mouse cell, it has great difficulty copying itself because some viral genes don't work well in murine cells. "Clearly, there are blocks [to viral replication] that are very important, and I think they'll prevent the mouse from being an excellent model for AIDS pathogenesis," says Didier Trono of the Salk Institute for Biological Studies in La Jolla, California. Others are more hopeful. "With a bit of work, we may be able to overcome postentry replication restrictions," says the University of Pennsylvania's Doms, who is working with Frank Jirik of the University of British Columbia to make HIV-receptive mice. "It's well worth trying."

Mark Goldsmith of the Gladstone Institute of Virology and Immunology in San Francisco and colleagues hope to exploit the chemokine discoveries to create a different animal model for AIDS: a transgenic rabbit. Several years ago, NIAID's Thomas Kindt developed a transgenic New Zealand white rabbit that expressed human CD4 receptors. Although the animals did not develop disease, HIV could replicate more efficiently in their cells than in the mouse. Now, Goldsmith and others at the Gladstone have teamed up with Kindt to add chemokine receptors to these animals. "The challenge associated with rabbits is transgenesis methodology is substantially less efficient [than in mice] for reasons that aren't clear," says Goldsmith. Rabbits also have longer gestations, smaller litters, and nearly 20 times the housing costs of mice. Still, says Goldsmith, "we're optimistic."

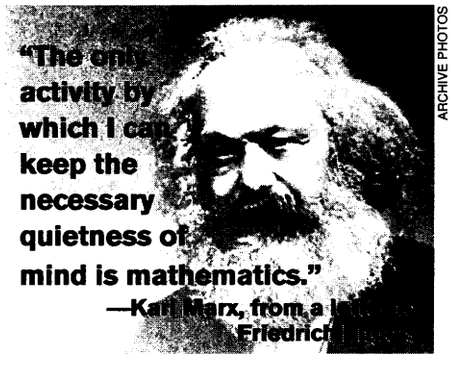
On every front, the revelation that HIV and chemokines have an intimate relationship holds an equal measure of promise and problems. But the gap between these basic studies and their application is narrowing fast.

–Jon Cohen

MATHEMATICS

In Mao's China, Politically Correct Math

SAN DIEGO—Karl Marx may be best remembered for inspiring the 20th-century revolutions in Russia and China. But during another upheaval, the Cultural Revolution in China during the 1960s and 1970s, his little-known musings on calculus may have saved mathematics.



ARCHIVE PHOTOS

According to Joseph Dauben, a historian of mathematics at the City University of New York, mathematicians in China seized on Marx's comments about "dialectical" processes in mathematics, along with related passages in the writings of Chairman Mao, to justify research activity that might otherwise have been denounced as a decadent, imperialist abstraction. They did so, Dauben said here at the joint meetings of the American Mathematical Society and the Mathematical Association of America, with the help of a highly abstract theory imported from the capital of Western imperialism, the United States.

The starting point for Dauben's account, which Chinese mathematicians corroborate, is Marx's own fascination with the interplay of thesis and antithesis—the dominant process in history, as he saw it—in certain mathematical procedures. Taking a limit, for example, entails thinking of a variable as both zero (thesis) and nonzero (antithesis—or possibly the other way around). "Marx regarded the analysis of the derivative, for example, as the analysis of a dialectical process, as the negation of a negation," notes Dauben. Mao, too, commented favorably on the ideological implications of mathematics, linking the "internal contradictoriness" of positive and negative numbers with the paramount importance of motion and incessant, revolutionary change.

So when revolutionary change threatened intellectual endeavors during the Cultural Revolution, Dauben says, mathematicians in China took refuge in research that arguably