Clue Found to T Cell Loss in AIDS

A "superantigen" encoded by the AIDS virus may cause the progressive immune cell depletion that leads to the collapse of patients' immune systems

IN THE NEARLY 9 YEARS SINCE THE AIDS virus was identified, researchers have picked it apart, urgently seeking to learn enough about its structure and complex genetics to find a way to beat it. Their progress in understanding the virus's molecular biology has been enormous—few viruses have ever been unraveled as rapidly as this one. But all this progress has yet to crack the central mystery of AIDS: How does HIV cause the immune cell loss that eventually robs patients of the ability to fight off infections?

It's not that AIDS researchers haven't had their theories. There have been many, such as the possibility that HIV needs help from another pathogen or triggers an autoimmune reaction that destroys the immune cells. But not one of the theories has been proved, or even disproved, to everyone's satisfaction—despite the high stakes. Would a better understanding of T cell depletion have an impact on efforts to develop AIDS vaccines and therapies? "Absolutely," says Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID).

Now, a research team at the University of Brescia, Italy, has provided a new clue to the immune cell depletion. The work comes on the heels of reports from several labs in recent months suggesting that some pathogens, including both viruses and bacteria, produce proteins that act as double-edged swords in the immune system. Called "superantigens," these proteins initially produce a massive stimulation of immune cell activities—but ultimately they lead to the cells' dysfunction and death. Could that be a mechanism of AIDS? According to the Brescia group's results, which appear on p. 860 of this issue of *Science*, the answer might be yes: HIV may be one of the pathogens that carries superantigens. "The paper is really intriguing because it provides one of the first clues that superantigens might be involved in AIDS," says immunologist Herbert Morse of NIAID, who studies an AIDS-like disease in mice.

But while the Brescia group's results are "intriguing," it's too soon to say whether the superantigen theory will win out over the many others that have been proposed. Indeed, the suggestion made several years ago, that HIV provokes the immune system into attacking itself, has also gleaned new support recently (see box on p. 799).

The reason that AIDS researchers have been having so much trouble understanding immune cell depletion boils down to two straightforward, but seemingly contradictory, observations. The inability of AIDS patients to respond to many pathogens is caused by the gradual disappearance of essentially all members of a particular class of immune cells: the "helper" T cells (also called CD4 cells because they carry a surface protein known as CD4), which are needed to initiate responses to the pathogens. But—and this is a major but—the loss of the CD4 cells apparently can't be accounted for by the direct cell-killing effects of the AIDS virus, since only a very small proportion, perhaps as few as 1 in 10,000, are infected.

That discrepancy has opened up all sorts of possibilities—the most extreme being the contention of Peter Duesberg of the University of California, Berkeley, that HIV isn't even the cause of AIDS. Few AIDS researchers buy Duesberg's hypothesis, but the evidence has forced them to come up with mechanisms by which HIV might kill helper cells indirectly. Five or 6 years ago, for example, William Haseltine, Joseph Sodroski, and their colleagues at Harvard's Dana-Farber Cancer Institute noted that HIV-infected T cells grown in lab culture can fuse with uninfected cells to form clumps of nonfunctional cells called syncytia. They proposed that syncytia formation could be a way that a single infected cell could cause the death of many uninfected cells.

Understanding T cell depletion would "absolutely" have an impact on efforts to develop AIDS therapies.

Alternatively, other researchers, including Luc Montagnier of the Pasteur Institute, who was the first to isolate HIV, have proposed that the virus doesn't work alone, but that "cofactor" pathogens, such as mycoplasmas, have to help it out. Still other researchers have suggested that HIV triggers an autoimmune attack that could kill uninfected cells. None of the theories has been able to win general acceptance, however.

Enter Luisa Imberti, Daniele Primi, and their colleagues at the University of Brescia, who decided to see whether superantigens might be at work in AIDS. They got the idea, Primi says, after seeing a series of results that came out earlier this year. One set, coming from four different labs, appeared in the 11 February issue of *Nature*, and collectively showed that mouse mammary tumor virus encodes a superantigen that can cause the deletion of specific subsets of T cells in mice.

But that wasn't the only signpost pointing in the direction of superantigens. NIAID's Morse and his colleagues found that a virus that causes an immunodeficiency syndrome in mice also encodes a superantigen (also see *Science*, 19 April, p. 424). The results prompted immunologist and superantigen expert Charles Janeway to suggest in a "News and Views" accompanying the *Nature* papers that a superantigen encoded by HIV (which like the two mouse viruses has an RNA genome) might contribute to AIDS pathology.

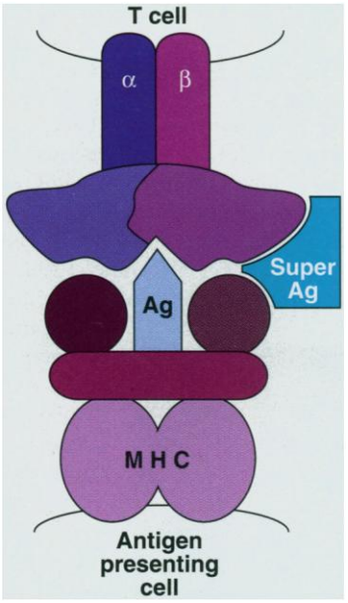
In beginning their work, Primi says, the Brescia group set out to look for the characteristic signature of superantigen action in AIDS patients. That signature takes the form of "holes," or gaps, in an individual's repertoire of T cell receptors. The receptor is essentially the T cell molecular trigger, setting off the cell's activities by recognizing and binding to an antigen, whether of the ordinary or super variety. But superantigens don't bind to the receptor the same way ordinary antigens do, and the difference can have fatal consequences for the cell.

Each T-cell receptor consists of two chains, designated alpha and beta. There are many variants of each, which can pair up in any combination, giving rise to a large number of possible receptors. Recognition of ordinary antigens requires both chains, and as a result, the recognition is very specific, resulting in the activation of only a handful of T cells. In contrast, a superantigen doesn't bind to the

normal antigen recognition site, but attaches on the outside surface of only one subunit—the beta chain. Consequently, a superantigen activates all T cells having the same beta chain variant, no matter what kind of alpha chain they have. The result: a far more massive response than ordinary antigens produce.

What's more, CD4 cells stimulated by a superantigen behave differently than those stimulated by ordinary antigens, eventually losing their ability to respond to antigens and disappearing from the circulation. The result will be detectable "holes" in the T cell repertoire.

That pattern suggested an experiment to Imberti, Primi, and their colleagues: They compared the T cell repertoires of six AIDS patients with the repertoires of six uninfected controls. The result was compatible with superantigen action. Alpha chain repertoires were the same in both groups, but the beta chain repertoires of the AIDS patients showed selective loss of some variants. The researchers think that HIV, rather than one of the many pathogens that cause opportunistic infections in AIDS patients, is the source of



The superantigen difference. *Both the alpha and beta chains of the T cell receptor bind to a normal antigen (Ag) presented on a major histocompatibility protein (MHC), but only the beta chain binds a superantigen.*

the superantigen, because there was no correlation between those infections and the T cell deletions. They found, for example, that HIV-infected people who had low CD4 cell counts, but who had not yet developed opportunistic infections, showed the same pattern of T cell deletion as the patients with full-blown AIDS. Primi cautions, however, that while the evidence for an HIV-encoded superantigen looks promising, it's still indirect. Definitive proof will require identifying the superantigen.

Still, new results independently obtained by three groups, those of Jean-Claude Ameisen and Andre Capron at INSERM's lab at the Pasteur Institute in Lille, France; Frank Miedema at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service in Amsterdam; and Montagnier at the Pasteur Institute in Paris, are at least consistent with the possibility that an HIV superantigen might contribute to T cell deletion in AIDS. These researchers have found that when T cells from AIDS patients are stimulated by antigens in lab culture, they don't proliferate the way normal T cells

Autoimmunity Explored in AIDS Pathology

The idea that the immune cell depletion seen in AIDS patients might be caused by an HIV-encoded superantigen isn't the only potential explanation of that phenomenon that has been getting attention recently. One of the older theories, namely that HIV tricks the immune system into attacking itself, has gotten a shot in the arm, partly because of a report in the 6 September issue of *Science* by Tracy Kion and Geoffrey Hoffmann of the University of British Columbia in Vancouver. "Now with that paper, [the autoimmune theory] becomes a viable possibility," says immunologist Gene Shearer of the National Institute of Allergy and Infectious Diseases, an early proponent of the theory.

The Kion-Hoffmann paper also gained a bit of undeserved notoriety, however, when *Nature* editor John Maddox cited it in an editorial as evidence in favor of Peter Duesberg's idea that HIV doesn't cause AIDS. This contention drew sharp criticism in AIDS circles as no one—not even Duesberg himself—was buying the Maddox argument (also see *Science*, 18 October, p. 376).

What Kion and Hoffmann's work did show was that mice immunized with lymphocytes from another mouse strain make antibodies to the HIV envelope protein gp120, as do autoimmune strains of mice, even though none of the animals had ever been exposed to the AIDS virus. One implication of the results is that some component on the lymphocytes resembles gp120 closely enough so that antibodies directed against it can recognize gp120 as well. The converse, of course, is that antibodies to gp120 should also recognize the lymphocyte component, so that an immune response directed against HIV might also knock out normal lymphocytes.

What might the lymphocyte component be? The histocompatibility proteins, which are normal participants in immune cell interactions as well as the targets for immune system rejection of transplanted tissue, or perhaps proteins that resemble the histocompatiblity proteins, are good candidates. Working from the known amino acid sequence of gp120, two other long-time proponents of the autoimmune theory, Angus Dalgleish of St. George's Hospital in London and John Habeshaw of Royal London Hospital Medical College have modeled the three-dimensional structure of a segment of the envelope protein. Their conclusion: That it resembles the structure of a portion of the major histocompatibility proteins.

Dalgleish and Habeshaw propose then that gp120 on the surface of infected cells could induce an immune attack on uninfected cells similar to that occurring in graft versus host disease. Indeed, Shearer says, the parallels between graft versus host disease and the immune system abnormalities in early AIDS are striking. Both feature, for example, a decline in the activity of CD4 cells but an increase in the activity of the other major T cell class, the CD8-positive killer cells. The autoimmune theory is also consistent, incidentally, with new results in showing that there is selective loss of particular subsets of T cells in AIDS patients (see p. 860), although the mechanism of the loss would be different in the two cases.

Kion and Hoffmann's view of how HIV infection produces an autoimmune response is more complicated than that of Dalgleish and Habeshaw since it features disturbances in two types of immune responses, one directed against the helper cells and another directed against a set of immune cells, known as suppressors, that stabilizes the helpers. Hoffmann views the whole system as a tent, he says, in which the helper cells are the canvas and the suppressor cells are the center pole. In HIV-infected people, he concludes, the immune system ends up "attacking both the canvas and the center pole." There are several other views of what causes the T cell loss, however, and it remains to be seen which will win out. ■ J.M.

would. Instead, says Ameisen, "The cells are primed to die when they are stimulated."

And it's not just any kind of death. They succumb to "apoptosis," which is also known as programmed cell death. Since superantigens are known to induce programmed cell death in CD4 cells, Miedema says, the finding of apoptosis suggests that they could account for the selective loss of the cells in AIDS patients. He notes, however, that opportunistic pathogens might be a source of the superantigens, as well as HIV itself.

The priming of T cells for programmed cell death could also explain another aspect of AIDS that's just beginning to be appreciated. "Most people didn't realize that before you lose a lot of T cells, the immune system is already dysfunctional," Miedema points out. And it certainly would be if helper cells die when they encounter antigens. In addition, stimulation of programmed helper cell death might be one way that cofactor pathogens could contribute to AIDS development.

What's still unclear, however, is how HIV infection might prime CD4 cells for programmed death. The researchers have suggested several possibilities. The viral infection might, for example, cause disturbances in the production of the many molecules that regulate immune cell activities, causing an aberrant response. Or the HIV envelope protein gp120, which has already been shown to impair T cell function, might be involved.

Nobody expects a definitive answer on just what kills T cells in AIDS patients any time soon, but when it comes the information should, as Fauci says, help in the design of better therapies. If drug therapies can't eliminate the AIDS virus from the patients' bodies, then it may at least be possible to mitigate its devastating effects on the immune system. But the current work suggests that therapy needs to begin early since the immune cell abnormalities start early. As Miedema puts it, "Once you lose a lot of T cells, you are already over the hill."

The information is also important for vaccine development. "People are trying to develop vaccines using virus proteins," Primi says. "But suppose you inject into people a protein with superantigen properties. The damage could be big."

And then there's the strong possibility of a major complication. According to the researchers, HIV may well cause T cell depletion in more than one way. "Everything about the virus is bad," says Janeway. "It has adopted every mechanism previously known for evading the immune system." And that makes the virus an extremely formidable adversary. ■ JEAN MARX

When Diamonds Met Buckyballs

Synthetic diamond coatings have become a superstar of materials science. Superstrong and superhard, they should be just the thing for armoring the business ends of drills, mining equipment, machine tools, even kitchen knives and razor blades; meanwhile, other properties have caught the eye of microelectronics and optics researchers. In fact, it was all this promise that led *Science* to name diamond films "The Molecule of the Year" last year (21 December 1990, p. 1640). But there was always a catch: The films won't grow from the parent carbon-containing vapor unless the surface to be covered has been pretreated with a polish of diamond grit—an impractical requirement, in many cases. Now salvation may be on its way from another superstar material, the cage-shaped carbon molecules called buckyballs. In the world of materials science, this mating must be the equivalent of movieland's marriage of Liz Taylor and Richard Burton.

Well met. *A 0.2-millimeter C_{70}-coated spot hosts a film of diamond* (bottom).

In a forthcoming issue of *Applied Physics Letters*, Northwestern University researchers R.P.H. Chang and Manfred Kappes and their colleagues report that diamond films grow eagerly on a specially prepared layer of C_{70} clusters—a relative of the original buckyball, C_{60}. That result should enhance not only the luster of diamond films but the box-office value of buckyballs. Since the discovery of the molecules in 1986, they have resembled unemployed celebrities. Oh, there's been plenty of promise—widespread speculation in the trades about possible roles—but no scripts have materialized. Which is why this first hint of a practical application has cheered buckyball enthusiasts. "My guess is that this discovery will assist the development of practical applications," says Rice University chemist and buckyball codiscoverer Richard Smalley.

Before the Northwestern University team tried buckyballs, many other researchers had been searching desperately for an alternative to diamond-grit polish to encourage the growth of diamond films. But their casting was lousy. None of the substances tried, including pump oil and various cage compounds made of hydrocarbons, possessed two key qualities: stability—enough to withstand the high temperatures of diamond growth—and an intricate three-dimensional structure that could provide a template for diamond's molecular architecture.

About a year ago, Chang recalls, he realized that the famed carbon clusters might offer a solution. "I thought, 'These are real beauties,'" says Chang. "They are chemically inert, resilient compounds and they contain a lot of structure, a lot of dimensionality." As it happened, buckyballs became available in gram quantities at about the same time, opening the way for Chang and his colleagues to test his hunch.

The group deposited buckyballs on a surface and bombarded them with carbon and hydrogen ions, breaking open the cage structure and exposing some free ends of the buckyballs' carbon network. These free ends, the Northwestern researchers theorized, would provide ideal templates for nucleating diamond growth. Their first effort to grow films on layers of C_{60} met with only limited success, but the C_{70} stand-in, with its slightly different geometry, made buckies look great. A base layer of ion-activated C_{70} was about 10 orders of magnitude better at seeding diamond-film growth than an untreated surface.

That's fast enough for practical diamond-film growth, says Chang, but he and Kappes are eager to see whether other buckyball relatives might do even better as stand-ins. They're now trying larger carbon clusters, such as C_{84} and C_{90}, as well as fragments of such clusters. On theoretical grounds, though, they think the ideal molecule may lie in an even larger size realm.

These material matchmakers, it seems, won't rest until they find diamond's perfect partner—which is, of course, what Liz may have done, after repeated tries. Then again, perhaps no one will ever equal Richard. ■ ANNE SIMON MOFFAT