AIDS RESEARCH

Cytokines Move From the Margins Into the Spotlight

REIMS, FRANCE—With neither a vaccine nor a cure in sight, the fight against AIDS has lately produced little worth uncorking a magnum of champagne over. Hence this city in the heart of the Champagne country might not seem the most appropriate place for a major meeting on the disease. But in fact, the mood was upbeat among the 275 researchers who gathered here last month* to review the role cytokines (proteins that act as messengers between cells of the immune system) might play in AIDS.

The Reims meeting itself was cause for celebration among some researchers. It was "the first time that cytokines have been considered as the front-liner" for an AIDS conference, says Jay Levy of the University of California, San Francisco (UCSF), a leading exponent of their role in the disease. And while many of the research findings are puzzling or controversial, a growing number of experts suspect that imbalances in the network of cytokines may ultimately trigger the immune system collapse caused by HIV.

Indeed, this view received a big empirical boost just 2 weeks before the meeting, when a team at the National Institute of Allergy and Infectious Diseases (NIAID) reported that in some AIDS patients, intermittent administration of a specific cytokine—interleukin-2 (IL-2)—dramatically boosted the number of CD4, or T helper, cells, the key immune cells that are lost as the disease progresses (*Science*, 3 March, p. 1261).

Despite this encouraging result, Thomas Folks, head of the Retrovirus Diseases Branch at the Centers for Disease Control and Prevention (CDC) in Atlanta, cautions against assuming "that we are going to find a major cure or correction in disease progression very rapidly." Nevertheless, says Folks, "I believe we are on the right road" by focusing on "cytokine pattern changes." And there was general agreement at the Reims meeting that there are now enough laboratory data on cytokines to justify expanding therapeutic trials.

This new focus represents a marked shift in the community of AIDS researchers. During the first decade of AIDS research, cytokines rated little more than an honorable mention. The turning point came about more than 2 years ago, when Mario Clerici and Gene Shearer of the U.S. National Cancer Institute offered a provocative hypothesis: that a shift in cytokine patterns might explain why HIV-infected individuals finally lose their battle with the disease (*Science*, 8 October 1993, p. 175). Although many researchers have come to regard this model as an oversimplification, it was very valuable, because it "has stimulated a lot of research," according to Jean-Claude Ameisen of the Pasteur Institute in Lille, France.

Clerici and Shearer proposed that the ability to fight off HIV depends on the balance between two subsets of T helper cells—designated Th1 and Th2—whose existence had been demonstrated by other workers. Th1 cells secrete the cytokines IL-2 and gamma interferon (IFN-γ), which are involved in stimulating the "cell-mediated responses" that help the body eliminate cells infected with pathogens. Th2 cells, in contrast, secrete interleukin-4 (IL-4), interleukin-10 (IL-10), and other cytokines that activate antibody production. In Shearer and Clerici's view, the Th1 cells are crucial to fighting off HIV; Th2 cells are less helpful, perhaps even harmful. When they measured cytokine secretion of T cells from AIDS patients, they found a steady shift from Th1 to Th2 patterns as the disease progressed.

While a number of laboratories have documented that HIV-infected individuals show alterations in cytokine patterns compared with uninfected controls, few researchers have been able to confirm that a clear-cut Th1/Th2 shift occurs as HIV infection progresses from the asymptomatic stage to full-blown AIDS—the central tenet of the Shearer-Clerici hypothesis. But Clerici and Shearer argue that these differing results are due to variations in laboratory techniques. "It's mostly a question of how you measure the quantity of cytokines," says Clerici, now at the University of Milan in Italy. And, Clerici argues, even researchers who don't see a clear shift agree that Th1 cytokines help keep HIV at bay. "We are reaching a consensus about what [cytokines] correlate with protection in these patients," he says.

At the Reims meeting, Ameisen presented recent data from his own lab to support an alternative model—one that implicates cytokine imbalances in the death of T helper cells. He and his co-workers in Lille found that T cells from the blood of HIV-infected patients are highly susceptible to apoptosis, or programmed cell death, when activated to proliferate. They found, however, that apoptosis of the T cells could be prevented by adding antibodies that block the Th2 cytokines IL-10 and IL-4, or by adding interleukin-12 (IL-12), a recently discovered cytokine that stimulates maturation of Th1 cells.

SOME SELECTED CYTOKINES

Cytokine	Major sources	Major effects
IL-1	Monocytes, macrophages, other immune cells	Induces wide range of inflammatory and immune responses
IL-2	Th1 cells	Stimulates T cell proliferation and differentiation; stimulates B cell proliferation
IL-4	T cells, macrophages B cells	Induces differentiation of Th2 cells Induces differentiation of B cells
IL-10	T cells, macrophages	Inhibits monocyte/macrophage function Suppresses inflammatory cytokines Enhances B cell proliferation
IL-12	Macrophages, B cells	Stimulates differentiation of Th1 cells
TNF-α	Numerous cell types	Wide range of inflammatory and other immune responses

Recent evidence suggests that IL-12 levels may be low in HIV-infected individuals. If that is the case, Ameisen suspects, even normal levels of IL-10 and IL-4 may be able to trigger programmed destruction of Th1 cells. T cell survival requires "coordinated signaling," says Ameisen. In HIV-infected patients, Ameisen concludes, a lack of coordination could result in what he calls an "abortive Th1-to-Th2 shift": Th1 cells dying without the accompanying increase in the number of Th2 cells predicted by Clerici and Shearer.

At the Reims meeting, Clerici presented an update of the Th1/Th2 hypothesis that incorporates a role for apoptosis. Nevertheless, he and Shearer continue to argue that a cytokine shift over the course of HIV infection is the motor that drives this process.

Inflammatory proposal. Whether Shearer and Clerici are correct in the details of their model or not, another, perhaps even more fundamental, question will still need to be answered: If a disruption in the normal array

* First International Symposium on HIV and Cytokines, Reims, France, 15–17 March 1995.

of cytokines is responsible for disease progression in AIDS, how does HIV interact with the cytokine network to trigger this disruption? One clue may lie in tumor necrosis factor-α (TNF-α), a cytokine that fosters a wide range of inflammatory reactions in response to infection. The reason some researchers think TNF-α may be a key player in AIDS is the growing body of evidence indicating that a state of chronic inflammation may be necessary to maintain HIV infection.

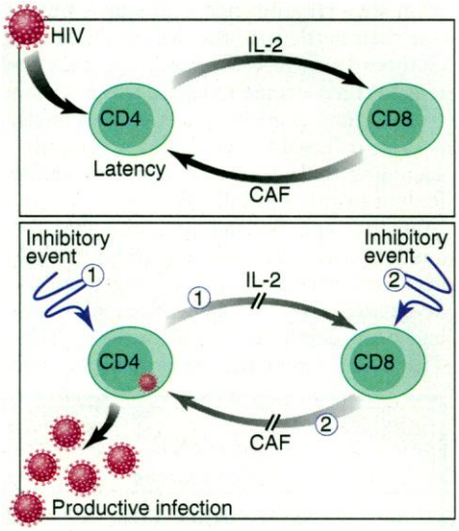
"Certainly in vitro," says Folks, "you need an activated, driven immune system to replicate the virus to high levels and to kill the cells." And that is where TNF-α may come in. This cytokine has been shown to greatly increase replication of HIV in T cells and other immune system cells called macrophages. TNF-α is thought to exert these effects by inducing a chemical factor in the cell nucleus, NF-κB, to bind to the viral DNA in the cell's nucleus and turn on the production of viral proteins.

In addition, several teams, including Anthony Fauci's at NIAID and John Hiscott's at McGill University in Montreal, have shown that the virus can also help create ideal conditions for its own growth: HIV, particularly in macrophages, can stimulate production of TNF-α and other inflammatory cytokines. Guido Poli of the San Raffaele Scientific Institute in Milan and other investigators are now turning their attention to the myriad of cell surface receptors through which TNF-α exerts its effects. For example, Salvatore Butera of the CDC presented evidence at Reims that HIV can stimulate some cells to increase their number of TNF-α receptors, making these cells more sensitive to the effects of the cytokine. "This is a very rich family" of receptors, says Poli, "and we are just at the beginning of understanding their role. I believe we have a spectrum of molecules that could be potentially useful for therapy or interference with the virus."

The fact that all immune cells secrete and respond to cytokines is also focusing attention on cells other than T helpers—and on the specific interactions those cells have with cytokines. For the past several years, a small band of scientists has been looking at the dendritic cell, a type of cell that is highly effective in stimulating T cells to respond to pathogens. At the Reims meeting, Stella Knight of St. Mary's Hospital Medical School in London reported that two cytokines that induce dendritic cells to mature—TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF)—also foster high levels of infection of dendritic cells in vitro.

Because only the mature forms of dendritic cells can activate T cells, Knight believes that the same cytokine environment that gets these cells ready to do their job at the same time makes them vulnerable to attack by HIV; one measure of their vulnerability is that she has found that mature dendritic cells from HIV-infected patients lose much of their ability to activate T cells.

But some other dendritic cell experts—while agreeing that these cells may be pivotal in AIDS—disagree with Knight about the extent to which they can become infected with HIV. For example, Ralph Steinman of Rockefeller University has found that when dendritic cells harboring small amounts of HIV are mixed with T cells in the laboratory, the two cell types fuse into "conjugates" that produce large amounts of virus. Steinman calls these results "ominous," because the normal close association of dendritic cells with T cells—a requirement for effective immune responses—may at the same time be "responsible for extensive and chronic viral replication."



Factored in. In a model developed by Jay Levy, the cytokine IL-2 stimulates CD8 cells to make a putative anti-viral factor, CAF, which keeps HIV under control in CD4 cells *(top)*. Blocking either CAF or IL-2 allows the virus to reproduce *(above)*.

Heroes and villains. If the dendritic cells' relations with cytokines help turn those cells into villains that victimize CD4 cells, there is increasing evidence that another member of the T cell family could turn out to be a hero—also as a result of its relations with cytokines. For almost 10 years, UCSF's Levy has been studying the effect of CD8 T cells on HIV infection. Although the main function of CD8 cells is to kill other infected cells, Levy has identified a subset of CD8s that strongly inhibits HIV replication in CD4 cells—without killing them.

Instead, Levy says, the CD8 cells release a cytokinelike substance, which he calls CD8 cell anti-viral factor, or CAF. Levy believes CAF inhibits transcription of the viral DNA from where it is perched among the cell's own genes—a necessary step in viral replication. Levy thinks production of CAF, which appears to decline as HIV infected patients progress to AIDS, may explain why some people can remain asymptomatic for years. Levy's work has never met with universal acceptance, however, partly because he has not been able to isolate the putative factor.

Nevertheless, other researchers are turning up data that support his hypothesis. Fauci, whose laboratory has recently been looking at CD8-mediated suppression of virus in the lymph nodes of HIV infected patients, says that "the points Jay made many years ago are correct. There's no question that CD8 cells can suppress HIV replication."

Rather than focusing on isolating Levy's CAF, however, Fauci has decided to look at the role other cytokines may play in regulating this effect. "We have clearly demonstrated," Fauci told *Science*, "that IL-2 is a potent inducer" of the CD8 phenomenon—a result consistent with Levy's own recent work, which indicates that the Th1 cytokine IL-2 enhances the suppressive effect, while the Th2 cytokines IL-4 and IL-10 inhibit it.

Both Levy and Fauci believe these results may shed light on how IL-2 was able to boost CD4 cell numbers in the recent therapeutic trials. Whether or not their explanation turns out to be correct, the CD8 story shows how high the stakes in the cytokine game could be. With lives at stake, AIDS experts are not waiting to understand the exact mechanisms of cytokine action before launching new clinical trials. According to Steven Schnittman, assistant director for clinical research at NIAID's AIDS division, the initial IL-2 trials are being expanded to include lower doses and subcutaneous rather than intravenous administration, to see whether side effects associated with higher doses—including fever and flulike symptoms—can be eliminated.

Another cytokine—IL-12—is also garnering considerable attention as a potential AIDS therapy. Given this cytokine's key role in fostering cell-mediated immunity, AIDS researchers are eagerly awaiting the start of two Phase II clinical trials—one in Europe and the other in the United States and Canada—which are expected to begin this fall. Dominique Emilie of the French medical research agency INSERM says that preliminary trials showed that IL-12 "seems to be very well tolerated, especially with subcutaneous treatment."

Schnittman cautions that evaluating the effectiveness of cytokine therapy may be difficult, because it is likely to be most effective in asymptomatic patients who still have intact immune systems. Nevertheless, says Poli, "after 10 years, anti-viral [drugs] have clearly not accomplished all we hoped they would. New approaches need to be taken, and I would say cytokines are the most likely candidates." That sentiment was widely endorsed at the Reims meeting, but nobody was arguing that it's time to start icing the champagne.

–Michael Balter

SOURCE: J.A. LEVY, *HIV AND THE PATHOGENESIS OF AIDS* (ASM PRESS, WASHINGTON, DC, 1994)