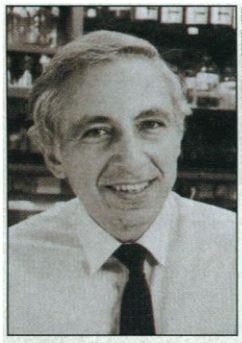
Elusive HIV-Suppressor Factors Found

Three factors, secreted by immune system cells, that apparently work in concert to suppress HIV replication have been identified after a long search; a fourth molecule may also be involved

ROME—HIV is infamous for outfoxing the immune system and ultimately destroying it. But researchers have long had hints that the immune systems of some HIV-infected people who don't progress to AIDS can outfox the virus. One of the strongest leads on what lies behind this ability has involved white blood cells known as CD8s: In 1986, virologist Jay Levy and researchers at the University of California, San Francisco, first reported that these cells can suppress the replication of HIV. And 3 years later, Levy and his colleagues demonstrated that the CD8s accomplished this feat by secreting an unidentified "factor" that potentially suppresses HIV's ability to copy itself. Yet no one had been able to identify this factor. Now, however, investigators have homed in on a trio of previously known immune molecules that they believe work in concert to produce the effects Levy first observed. If they're right, they not only will have solved a long-standing scientific mystery, they also will have paved the way for possible new treatments and new strategies to evaluate preventive vaccines.



NATIONAL CANCER INSTITUTE

Key factors. Team leaders Robert Gallo *(top)* and Paolo Lusso.

The new work comes out of the former National Cancer Institute lab of Robert Gallo, the co-discoverer of HIV and director of the new Institute of Human Virology at the University of Maryland. As Gallo briefly revealed at an AIDS meeting here last week,* a team headed by himself and Paolo Lusso, now at the San Raffaele Scientific Institute in Milan, Italy, found that three closely related polypeptides involved in inflammatory responses work together to produce the Levy effect. Known as RANTES, MIP1-α, and MIP1-β, these so-called chemokines shut down production of a broad range of strains of HIV-1, HIV-2, and SIV (the simian AIDS virus) that were growing in laboratory cultures. "There is something happening here," says Anthony Fauci, chief of the National Institute of Allergy and Infectious Diseases. "There is no question that this is door-opening time."

Gallo's abbreviated discussion of the finding followed a decision by *Science* to lift the embargo on a paper detailing the work, which will be published in the 15 December issue. *Science* took this action to coincide with the publication in the 7 December issue of *Nature* of a scientific correspondence, which *Nature* rushed into print, reporting a different—and apparently less powerful—HIV-suppressing factor that is also secreted by CD8 cells. Rumors of the Gallo-Lusso work were already widespread, and *Science* editors decided that it could potentially create confusion on a topic with clinical implications to keep the paper under embargo for another week.

For many scientists attending the Rome meeting—a small number of whom had been given embargoed copies of the *Science* paper—Gallo's news overshadowed the other highlights of the gathering, which included a dinner at the lavish palace of the Princess Pallavicini and a surprise visit by Mother Theresa. "The paper seems to be very convincing," says immunologist Sergio Romagnani of the University of Florence. "It provides direct evidence that the CD8 suppression of HIV is mediated by these chemokines."

Despite the considerable enthusiasm for the Gallo group's discovery, there is at least one voice of dissent—and it's coming from Levy. "I'm gratified that people are turning to look at what we have been working on," says Levy, who was reached by *Science* while on vacation in Brazil. "But if they say it's the factor I've been working on for the past 5 years, they've got the wrong ones." Levy, who says he had already screened for RANTES, MIP1-α, and MIP1-β as candidates for the elusive factor and ruled them out, nevertheless agrees that these chemokines may have promise as anti-viral therapy for HIV-infected patients.

Gallo says he is convinced that he and his co-workers have indeed identified Levy's factor, because they followed Levy's published protocols in preparing the CD8 cells used in their own experiments. What's more, as their paper shows, they abolished all of the HIV suppressor activity by adding to their cell cultures antibodies that specifically "neutralized" these three chemokines. Yet Gallo maintains that it "may be beside the point" whether these chemokines are the Levy factor. "This paper describes a major anti-HIV activity of CD8 cells," he says.

The brief scientific correspondence published in this week's issue of *Nature* by Reinhard Kurth, director of the Paul Ehrlich Institute in Langen, Germany, and his colleagues adds another layer of complexity to the picture. The Kurth group reports evidence that interleukin-16 (IL-16), a recently identified chemical messenger known as a cytokine, also suppresses HIV replication.

Kurth has long been investigating why African green monkeys do not become sick from SIV, which can quickly cause AIDS in Asian monkeys. The Levy factor had been a main suspect. Kurth's correspondence reports the isolation of nearly identical IL-16 molecules from humans and African green monkeys, both of which suppress HIV replication at relatively high concentrations in cell cultures. Levy also rejects this cytokine as a candidate for his factor, however, saying that his group eliminated IL-16 in recent experiments.

Fauci, who wrote a commentary in *Nature* to accompany Kurth's letter, told *Science* that it's possible that the Gallo group's factors and IL-16 may all play a role in HIV suppression. However, he said he found the Gallo group's results especially convincing because "Gallo is getting really impressive suppression at low concentrations [of chemokines], and in the Kurth paper the concentrations are much higher." Fauci adds: "They are both important papers. ... It is too much to ask that there is going to be one factor to the exclusion of all others."

The factor hunt

Levy first had a notion that there was something different in the CD8 populations of some HIV-infected people nearly a decade ago. CD8 cells are famous for acting like heat-seeking missiles that are directed at cells infected with HIV or other pathogens. But in 1986, Levy reported in *Science* another

* "AIDS and Related Diseases," Italian National Association Against AIDS, Rome, Italy, 30 November to 4 December 1995.

curious property of a subset of CD8 cells: They could strongly inhibit replication of HIV. And in 1989, he demonstrated that the inhibition was due to a soluble factor secreted by the CD8s. Interestingly, Levy found that the level of this HIV-suppressing activity appeared to decline as HIV-infected patients progressed to full-blown AIDS.

Yet for a number of years, Levy's work was regarded with widespread skepticism. "Years and years went by with no one paying attention," says Max Essex, head of the Harvard AIDS Institute at the Harvard School of Public Health. "My impression is that people didn't really believe it at all." The race for the factor began in earnest only within the last 2 years, when several leading AIDS researchers began to focus on the problem.

Lusso suggests that the sudden interest in the Levy factor was sparked by the modest results from AZT and other anti-viral therapies, as well as dimming hopes that there would be an anti-HIV vaccine any time soon. "I guess it was at this point that scientists started to realize that a simple, straightforward approach to therapy or vaccines was going to bring a lot of disappointment," he says.

Lusso says he believes his group succeeded where others have come up empty-handed because of two technical breakthroughs. One is the in vitro culture of a line of CD4 white blood cells—the main cell that HIV infects and destroys—called PM1. This PM1 line is easy to infect by fresh isolates of HIV taken directly from infected patients, as well as by strains that preferentially infect immune cells called macrophages. And, as compared with HIVs that have been passed through generations of laboratory cell cultures, these strains are the most sensitive to the CD8 suppressor factor.

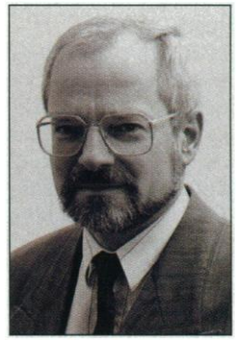
The other critical technical advance, says Lusso, is a new immortalized CD8 cell line developed by the Gallo group that pumps out high concentrations of the three chemokines. With enough material to work with, the team was able to concentrate the polypeptides in crude fractions and then further purify them using high-performance liquid chromatography.

DAVID POWERS

The Levy factor. Jay Levy provided first evidence for a suppression factor secreted by CD8s.

When the team sent the compounds to an outside lab for analysis of their amino acid sequences, they could not believe their luck: The sequences matched those of RANTES as well as MIP1-α and MIP1-β. But the critical experiments remained to be done. To prove that the three chemokines were in fact responsible for the suppressor effect, the group had to demonstrate two key points: First, that pure versions of the compounds could suppress viral replication when added to HIV-infected CD4 cells; and second, that the suppressor effect of supernatants of CD8 cells taken from HIV-infected individuals could be eliminated by the action of neutralizing antibodies directed against the chemokines.

The results of these experiments led to yet another eye-opener. The team found that although RANTES potently inhibited HIV production, MIP1-α and MIP1-β were somewhat less powerful. More troubling still, when antibodies to the three chemokines were added individually to the CD8 cell supernatants, they had very little effect. But when the researchers hit on the idea of using all three chemokines at once, they struck pay dirt. In the paper's most dramatic result, the researchers discovered that when they added neutralizing antibodies to all three chemokines at once, they were able to abolish all of the HIV suppressor activity of the CD8 supernatants.



Fourth factor. Reinhard Kurth found IL-16 also appears to suppress HIV.

Indeed, it is the group's antibody work that has most convinced other researchers of its validity. Immunologist Hans Wigzell, president of the Karolinska Institute in Stockholm, Sweden, says the results are "highly credible because they are at low, seemingly physiological, concentrations. From the point of view of scientific curiosity, it is a milestone. The hard thing is next—to see the clinical relevance in patients."

Moreover, the antibody studies may help to explain some of the discrepancies between the Gallo-Lusso paper and the Kurth group's letter in *Nature*, as well as at least some of the objections raised by Levy. Kurth told *Science* that in preliminary experiments he tested both RANTES and IL-16, but found that "RANTES didn't work and IL-16 did work." However, Kurth says, he tested RANTES only in a T cell lymphoma cell line infectible by HIV—a test system unlikely to be as sensitive as the Gallo group's specially designed PM1 cell line—and did not add the other two chemokines at the same time. "We will now put all four factors into the test system," he says. "Nobody at this stage knows to what extent the different factors contribute to HIV suppression."

Likewise, in the process of screening for known immunologically active molecules over the past several years—which involves the use of neutralizing antibodies to test for their activity—Levy says he cannot recall whether he tested for RANTES, MIP1-α, and MIP1-β at the same time. A number of researchers contend that if Levy only tested one chemokine at a time he might easily have concluded that they were not effective in suppressing HIV. Nevertheless, Levy maintains that he has seen no correlation between individual concentrations of these chemokines and HIV suppressor activity.

Another reason Levy doubts that these chemokines are the factor he has been hunting for is that they do not appear to act directly on the process of viral RNA transcription—the mechanism he has postulated as the primary explanation for how his factor suppresses HIV replication. Gallo and Lusso note that their factors do inhibit RNA transcription, but they believe that the block may come at an earlier stage of the virus' life cycle, before transcription begins.

"Levy has not proven that it is at the transcriptional level," Gallo says, although he concedes that Levy's results are "compatible" with transcriptional blockage. But a number of researchers say they are most intrigued by the fact that the three factors act together in some way. "It's extremely interesting," says Antonio Siccardi, coordinator of AIDS research programs at the San Raffaele Institute in Milan. "It makes me think about some kind of synergy."

However the factors exert their HIV-suppressing actions, AIDS researchers say these studies should be followed up quickly. "If I was the typical pharmaceutical company, I'd be looking at all of these," says Essex. "I wouldn't be sitting on my butt making one more derivative of AZT." At the moment, of course, the ability of these compounds to inhibit HIV has only been demonstrated in the test tube. And Gallo, for one, is circumspect about whether the chemokines will lead to new therapies. "There is no reason to believe that this is the Holy Grail," he says. Still, he and Lusso will soon launch toxicology studies in animals, with the ultimate goal of beginning human clinical trials at Gallo's new institute in Baltimore and at the San Raffaele Institute in Milan.

Marc Girard of the Pasteur Institute in Paris, a leader of the French AIDS vaccine effort, says it's too early to say whether these chemokines will have practical applications. But he, too, is optimistic. "You open a new window in the wall, and then you have to look through the window to see what you can see," says Girard. "There's a whole new landscape out there." And it's a landscape that many researchers are sure to begin exploring quickly.

–Michael Balter