AIDS RESEARCH

HIV 'Cofactor' Comes in For More Heavy Fire

AIDS research that makes big claims inevitably attracts commensurate attention—and criticism. But rarely is the attention and criticism as intense as that surrounding work reported last October at the Cent Gardes AIDS meeting by Ara Hovanessian of France's Pasteur Institute. In his talk, Hovanessian claimed that he and his coworkers had finally found the long-sought "cofactor" that helps HIV infect cells. It was, he said, a cell surface protein called CD26. If the finding holds up, it could lead to important new therapeutic strategies for AIDS and better animal models. But at the time, the claim touched off a firestorm of criticism from AIDS researchers who contended that Hovanessian had failed to make his case (*Science*, 5 November, p. 843).

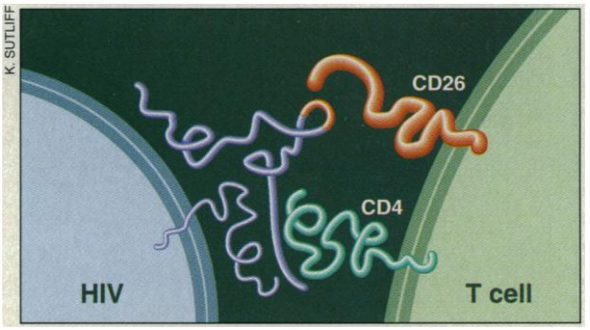
Now, a full description of the work has been published (see page 2045) and it's safe to say that the skeptics, many of whom have also investigated CD26's possible link to HIV infectivity, remain unconvinced. "The whole paper's based on a poor assay," says Joseph Sodroski of the Dana-Farber Cancer Institute. "Why would you expect good data out of a bad assay?" Thomas Schultz, a virologist at England's Chester Beatty Laboratories who studies the mechanisms HIV employs to enter cells, is also "very, very skeptical" that the results will prove true. "I just don't trust many of the experiments," says Schultz. "If you don't know how tricky some of these assays are, the paper looks more carefully done than it actually is."

Hovanessian also has his supporters, however. Pasteur's Luc Montagnier, who first isolated HIV in 1983, maintains that he is certain the work will withstand the scrutiny. "If each piece of data is taken separately, maybe it's not convincing," says Montagnier. "But taken together, they fit very well."

Hovanessian and Pasteur's Christian Callebaut, Bernard Krust, and Etienne Jacotot offer several lines of evidence that CD26, an enzyme also known as dipeptidyl peptidase IV, is a cofactor in HIV's infection of cells. In one experiment, for example, they tested whether monoclonal antibodies directed against CD26 would inhibit HIV's entry into a line of human T cells. The result: HIV's infectivity appeared to be markedly reduced when the cells were first treated with the monoclonal, which presumably binds to CD26 and blocks any interaction it might have with HIV. They saw similar results with IPI, an inhibitor of CD26's enzymatic activity.

To investigate CD26's role more directly, the investigators next introduced the gene for human CD26 into mouse cells, either by itself or together with the gene for another cell surface protein, known as CD4. Earlier work had shown that HIV binding to CD4 is necessary, but apparently not sufficient, for viral entry into cells. In the current work, the Hovanessian group found that, consistent with their hypothesis, HIV easily slipped into the mouse cells and replicated only when both CD4 and CD26 were present.

In order to measure virus entry into cells and viral production in these experiments, the Pasteur group first treated cells that had been exposed to HIV with the enzyme trypsin to remove external virus. This procedure, as well as Hovanessian's CD4/CD26 transfection method, came in for harsh criticisms at the Cent Gardes meeting. And the critics don't seem to be persuaded by the paper, either. "If they're right, they're to be congratulated," says Dan Littman, a Howard Hughes Medical Institute immunologist at the University of California, San Francisco (UCSF). "But they did it in a very unconventional way, to be sure."



Contested model. Does CD26 help the AIDS virus enter cells as the Hovanessian group thinks?

For one thing, many researchers are concerned that virus entry and production would be overestimated if the trypsin treatment does not destroy all the extracellular HIV. Hovanessian argues, however, that he did not see any sign of this problem with control cells. UCSF's Littman and other researchers also are concerned that the Pasteur group only transiently transfected cells with CD4 and CD26, which can lead to widely varying infectivity results. Instead, Littman says, Hovanessian should have made a stable transfected cell line. "*Science* should have asked him to do the definitive experiment," says Littman. "It would have taken him a month....On the balance of what's here, they may be right or they may be wrong."

Hovanessian has not yet been able to make stable transfectants, but at *Science*'s request he included other experiments with the anti-HIV drug AZT, which he says proves that the virus they were measuring had entered the cells and replicated. In these tests, they treated the CD4/CD26 transfectants with AZT, then tried to infect them with HIV. These cells, in contrast to controls not treated with the drug, did not produce virus. "How can you explain that AZT experiment?" asks Hovanessian. "[The replication] just cannot be an artifact. It cannot."

Though Quentin Sattentau, a viral immunologist at France's Centre d'Immunologie de Marseille Luminy, still has reservations about the results, he says the paper is vastly more informative than the Cent Gardes presentation. "I was rather dismayed after the talk at Cent Gardes and the paper clears up a lot of the anxiety," Sattentau says.

Still, since Hovanessian's talk, several groups have generated data indicating that CD26 does not help HIV get into cells. These researchers include Irvin Chen of the University of California, Los Angeles; Edward Berger of the National Institute of Allergy and Infectious Diseases; Michael Norcross and Tamas Oravecz of the Food and Drug Administration; and Sodroski of Dana-Farber. Hovanessian contends that these researchers were measuring cell-cell fusion, which does not necessarily reflect virus-cell fusion, or allowed multiple cycles of viral infection. Thus, he contends, comparing his work to theirs is misleading. "There's no problem reproducing our results," assures Hovanessian. "If we did the experiment the way others are doing it, it wouldn't work for us, either."

Why did *Science* accept a paper that is being so heavily attacked by Hovanessian's peers? "This paper was reviewed very thoroughly, by respected investigators within and outside of the HIV community," says *Science* senior editor Barbara Jasny. Indeed, after *Science* learned that one of the original reviewers may have had a potential conflict of interest, the paper was sent out for additional reviews.

If the CD26 finding is real, AIDS drugs might be developed that inhibited CD26 and prevented HIV from infecting virgin cells. And if a CD4/CD26 transfected mouse can be engineered, it would provide a much-needed small animal model. But for now, AIDS researchers remain to be convinced that Hovanessian is right. In fact, Chester Beatty's Robin Weiss, in a speech opening an AIDS conference in Washington, D.C. last week, noted that the paper was about to be published and issued a "caveat reader." Said Weiss: "Read *Science* with open eyes."

–Jon Cohen