



# Update

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## Soluble CD4 Linked to Toxin: Potential New AIDS Treatment

Working together, NIH scientists at the National Institute of Allergy and Infectious Diseases (NIAID) and the National Cancer Institute (NCI) have produced a recombinant protein called CD4-Pseudomonas toxin, that attaches to and kills cells actively producing the human immunodeficiency virus (HIV), the cause of AIDS. Previously, in the laboratory, soluble forms of CD4, the receptor by which HIV enters cells, have shown promise for treatment of AIDS by preventing the virus from entering cells. The first clinical trials of CD4 began last month.

In this new development, NIH scientists collaborated to link soluble CD4 to a toxin. The researchers are hopeful that CD4-toxin will have a therapeutic effect in AIDS patients by eliminating HIV-infected cells that otherwise would produce more virus as well as potentially harmful viral products. The laboratory findings look promising, but the scientists caution that further laboratory and development work remain before studies with patients can begin.

To gain entry into human cells (which the virus must do to replicate, or multiply), HIV attaches to CD4, a receptor found on the surface of certain cells, including critical immune system cells. The surface, or envelope protein of HIV, called gp120, binds to CD4 on a cell and then the virus enters the cell. Once infected, the cell becomes an HIV factory, manufacturing all of HIV's components including gp120. As the gp120 collects on the infected cell's surface, new viruses assemble and leave the cell. These new viruses can then spread HIV infection to healthy CD4 containing cells. In addition, the gp120 on the surface of an infected cell can cause the cell to attach to and fuse with uninfected cells that have CD4 receptors, thus providing another route for spread of HIV infection in the body.

Paving the way for the currently reported findings, LVD scientists and others have genetically engineered soluble CD4 that attaches so tightly to gp120 on the virus or to gp120 on the surface of the infected cell, that it prevents gp120 from binding to natural CD4 on healthy cells. Scientists theorize that soluble CD4, by acting as a "decoy," might limit spread of infection in HIV-infected people.

In order to lessen chances that laboratory-made soluble CD4 would interfere with natural CD4 function, scientists wanted to use only the smallest portion of cellular CD4 to which HIV attaches. In June, at the Fourth International Conference on AIDS held in Stockholm, Dr. Edward A. Berger, senior scientist, LVD, reported that he and his colleagues Dr. Tamio Mizukami and Dr. Thomas Fuerst had identified the portion of CD4 to which HIV attaches.

The researchers accomplished this by analyzing variants of the natural CD4 protein. These variants were produced by genetic engineering techniques using a CD4-producing vaccinia virus system developed under the direction of Dr. Bernard Moss, chief, LVD.

Can cells already infected with HIV be eliminated from the body using soluble CD4 technology? The NIAID scientists considered that by linking soluble CD4 to another protein that is toxic to cells, the CD4 component would target the toxin to seek out and kill HIV-infected cells with gp120 on their surfaces. Healthy uninfected cells with no gp120, would be spared.

With their CD4 data in hand, the LVD scientists turned to their colleagues at NCI. Dr. Ira Pastan, and Dr. David FitzGerald of NCI's Laboratory of Molecular Biology have been developing modified toxin conjugates as therapeutic agents for cancer. Using a variety of techniques including genetic engineering, the NCI scientists have linked all or portions of a bacterial toxin called Pseudomonas exotoxin A to a variety of targeting proteins including monoclonal antibodies that are tailored to deliver the toxin to and kill specific cancer cells. These "targeted toxins" are currently in clinical trials at NIH with ovarian cancer patients.

In the newly reported CD4-toxin research, NCI's Dr. Vijay Chaudhary, a senior scientist in Dr. Pastan's laboratory, genetically engineered the bacterium E. coli to produce a hybrid protein called CD4(178)-PE40 (CD4-Pseudomonas toxin). This protein contains the portion of CD4 that attaches to HIV gp120 linked to the portion of the toxin that kills cells. Because it binds to gp120 on the surface of infected cells, CD4-toxin acts like a self-guided missile that searches out and destroys only cells that are infected with HIV and actively producing gp120. The scientists have demonstrated highly selective killing of HIV-infected cells in culture, suggesting that this novel derivative of soluble CD4 may prove to be a potent weapon for treatment of AIDS.

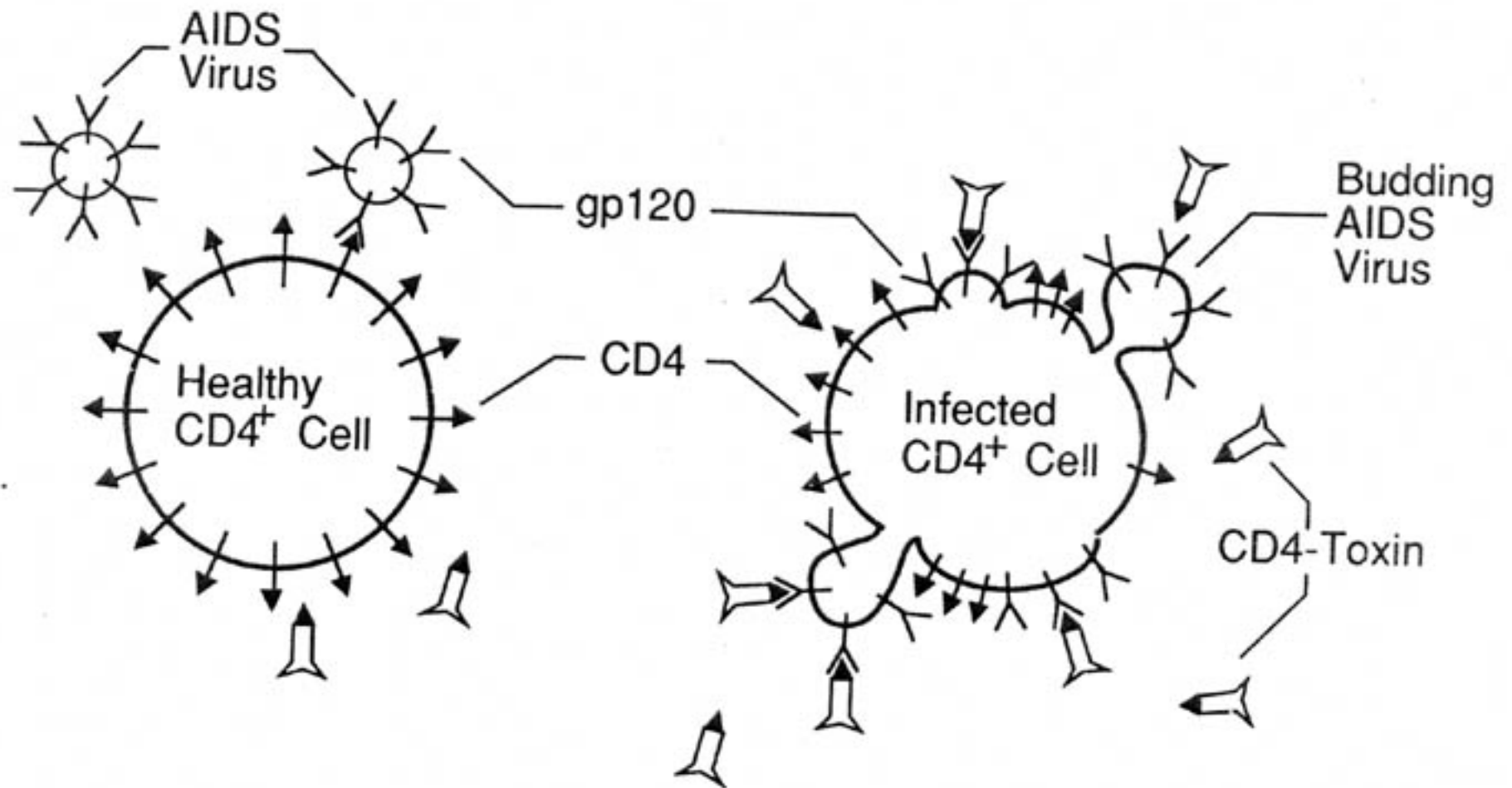
Future work will include continued testing of the effect of the soluble CD4-toxin recombinant protein on different types of normal and HIV-infected cells in cell culture. One question is whether all HIV-infected cells have gp120 on their surfaces and will be targeted. The researchers are currently working to obtain larger quantities of the recombinant CD4-toxin protein that will be necessary for testing in animals and humans.

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These findings are published in the September 22, 1988 Nature 335:369-372 in an article titled "Selective Killing of HIV-Infected Cells by Recombinant Human CD4-Pseudomonas Exotoxin Hybrid Protein." The authors are Vijay K. Chaudhary (1), Tamio Mizukami (2), Thomas R. Fuerst (2), David J. FitzGerald (1), Bernard Moss (2), Ira Pastan (1), and Edward A. Berger (2).

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The gp120 on the AIDS virus recognizes CD4 on the healthy cell (left). This allows the virus to attach to and enter the cell. The infected cell (right) becomes an AIDS virus factory, manufacturing all virus components including gp120, which accumulates on the cell's surface. New viruses bud off (leave) the infected cell.

CD4-*Pseudomonas* toxin is a hybrid protein designed by genetic engineering specifically for treatment of AIDS. It contains a portion of CD4 linked to a portion of bacterial *Pseudomonas* toxin. Because the CD4 portion of the CD4-toxin can attach to gp120, CD4-toxin acts like a self-guided missile that searches out and destroys only cells infected with the AIDS virus. The killed cells no longer produce new virus or harmful cell products. Healthy cells not infected with the AIDS virus have no gp120 and therefore are not killed by CD4-toxin.