HIV Infection: The Cellular Picture

A key finding of AIDS research is that infection begins when HIV binds to a molecule called CD4 on the target cell. Knowledge of that interaction may help in developing therapies or vaccines

by Jonathan N. Weber and Robin A. Weiss

Like all viruses, the human immunodeficiency virus (HIV) is an intracellular parasite: the virus particle itself is inert and cannot propagate or do any damage until it enters a host cell. How does the virus actually enter the cell? The answer will help investigators to understand the clinical course of AIDS, the disease caused by the virus. More than that, an understanding of how HIV enters cells may eventually make it possible to develop vaccines or protective medications that can block the action of HIV at the earliest possible stage: before it infects its first host cells.

The first step in any viral infection is the binding of the virus particle to a component of the host cell's membrane. In the case of HIV, workers have found that the virus binds to the molecule known as the CD4 antigen. (An antigen is a molecule that can be recognized by an antibody.) Hence the distribution of CD4 in the body reflects the tropism of HIV: the kinds of cells and tissues the virus infects and destroys. The CD4 antigen is found primarily on cells of the immune system called helper *T* cells (although other kinds of cells also carry it); HIV infection is characterized by the loss of these cells, which causes a deterioration of the immune system.

For some time it has been known that the binding takes place when CD4 interacts with an "envelope" protein of the virus called gp120 (because it is a glycoprotein—a protein containing sugar complexes—with a molecular weight of 120 kilodaltons) that is distributed on the outside of the viral membrane. Investigators are now identifying the specific portions of the CD4 and gp120 molecules that take part in the binding interaction. Such knowledge makes it possible to envisage a two-pronged attack on HIV: denying access to the cellular CD4 receptor, both by covering up the viral gp120 protein and by blocking the receptor.

The chain of experiments that eventually identified CD4 as the molecule to which HIV binds began in June, 1984, when samples of the virus became generally available for research. In one of the earliest experiments, Mika Popovic of the National Institutes of Health studied the growth of HIV in fresh peripheral-blood lymphocytes (white blood cells freshly separated from the bloodstream) and in lines of tumor cells that are able to grow perpetually in culture. He found that HIV grew best in a line of leukemic *T* cells. (The *T* cells, a major class of cells in the immune system, include the helper *T* cells and cells called cytotoxic, or killer, *T* cells.)

At about the same time, David Klatzmann of the Salpêtrière Hospital in Paris noted that in fresh peripheral-blood lymphocytes infected in culture with HIV there was a decrease in the number of cells bearing the CD4 antigen; the decrease was paralleled by an increase in the HIV replication rate. Klatzmann then divided the *T* cells from a sample of peripheral-blood lymphocytes into *T*-helper and *T*-cytotoxic subsets. He found that only helper *T* cells—the cells that bear the CD4 antigen—supported the replication of HIV. Klatzmann's findings dovetailed well with an observation made in 1981 in the first published clinical description of AIDS patients. In that report Michael S. Gottleib of the University of California at Los Angeles School of Medicine had noted that lymphocytes bearing CD4 were reduced in number or absent entirely from the blood of AIDS patients.

Simultaneously in London, Angus G. Dalgleish and Paul R. Clapham in our laboratory at the Institute of Cancer Research tackled the question of the tropism of HIV from another direction. We tested antibodies to various cell-surface antigens to see which of them would block molecules crucial to the binding of the virus. In these experiments we first exposed susceptible *T* cells to the antibodies and then to virus particles. Next we applied various assays to determine how the antibodies had affected HIV's ability to infect the cells. These experiments revealed that monoclonal antibodies (antibodies that bind only to a single, specific molecular target) to the CD4 antigen, but not those to other cell-surface antigens, could block the infectivity of HIV. Klatzmann, using different assays, got similar results.

Another kind of assay took advan-

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tage of a sign of HIV infection we had noted in cell cultures: the formation of "multinucleated syncytia." These are giant cells consisting of several nuclei contained within a single membrane; they form when HIV-infected cells fuse with healthy cells bearing the receptor molecules. We found that antibodies to CD4 could indeed block the formation of syncytia.

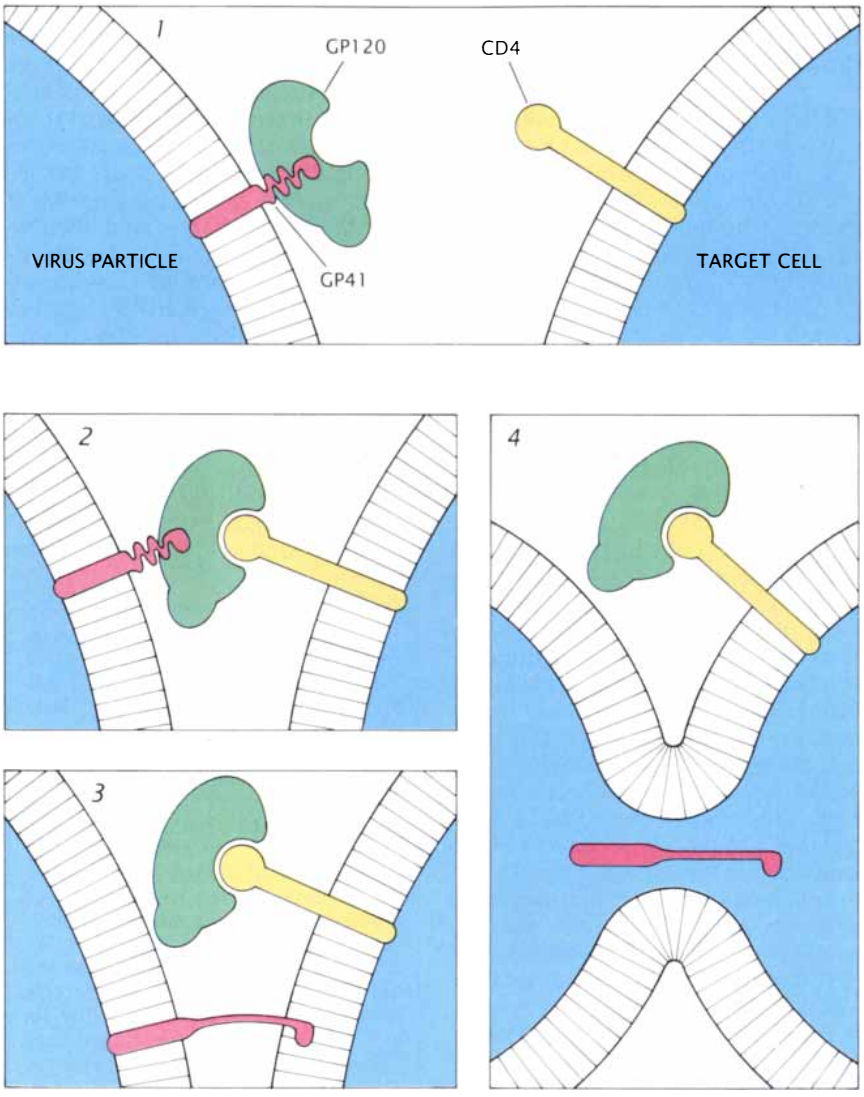
Still another assay for receptors, first developed for work on animal retroviruses by Jan Zavada of the Institute of Virology in Bratislava, is known as a pseudotype assay. This method involves exposing cells that have already been infected with HIV to a second, unrelated virus called vesicular stomatitis virus (VSV). VSV is a plaque-forming virus: it causes the formation of visible plaques made up of dead cells. When HIV-infected cells are "superinfected" with VSV, they produce a number of virus particles that have the envelope proteins of HIV but the genetic material and plaque-forming properties of VSV. These "transvestite" particles are called VSV-(HIV) pseudotypes. Because the pseudotypes have the same envelope characteristics as HIV, they recognize the same receptors and enter the target cell in the same way; their ability to infect particular cells should therefore parallel that of HIV. After they enter the cell, however, they replicate as VSV and form plaques. Hence the appearance of plaques in various kinds of cells indicates the presence on the surface of those cells of the receptor for HIV.

Dalgleish and his colleagues noted that VSV(HIV) pseudotypes would form plaques only among cells bearing the CD4 antigen. Furthermore, the antibodies to CD4 that blocked the formation of syncytia also prevented the formation of plaques.

Subsequently J. Steven McDougal of the Centers for Disease Control in Atlanta (CDC) devised a physical assay for determining whether HIV particles had attached to cells; he found that HIV would bind only to cells bearing the CD4 antigen and, once again, that binding could be inhibited by anti-CD4 monoclonal antibodies. McDougal also showed that gp120 molecules attached to antibodies could draw CD4 molecules from a preparation of cell-membrane material. All these experiments suggested that the CD4 antigen—the disappearance of which had been part of the clinical definition of AIDS from the disease's earliest days—is itself the receptor for HIV.

The strongest evidence that CD4 is the receptor for HIV came in 1986 from Paul Maddon and Richard Axel of the Columbia University College of Physicians and Surgeons. They transferred the gene that encodes the CD4 molecule into HeLa cells, a line of cervical-cancer cells that do not make CD4 and cannot ordinarily be infected with HIV. Maddon and Axel found that the altered, CD4-bearing HeLa cells could now be infected with HIV; when they were infected, they rapidly fused into giant syncytia. Expression of the CD4 gene was enough to confer susceptibility to HIV.

This experiment led to one unexpected result, which has not yet been explained fully. Maddon, working in collaboration with Clapham and Dalgleish in London and McDougal at the CDC, transfected the human CD4 gene into mouse *T* cells; the cells then produced human CD4. HIV particles bound to these altered cells, but there was no evidence that the cells actually became infected: no syncytia were formed and no infectious virus was produced. This was surprising, because mouse cells can indeed produce HIV under certain conditions; for example, Jay A. Levy of the University of California at San Francisco School of Medicine and other investigators successfully transfected the entire HIV genome into mouse cells, which then produced infectious virus. Apparently, however, mouse cells cannot be infected by free HIV particles, even in the



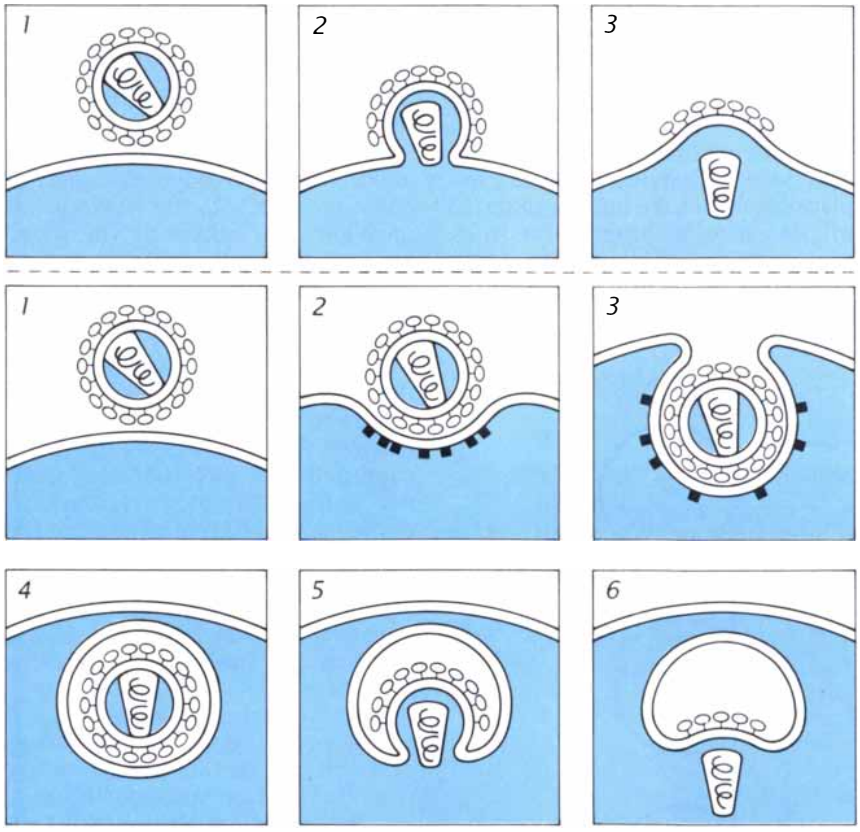
BINDING of a virus particle to a target cell depends on an interaction between a molecule on the surface of the virus and a molecule on the membrane of the target cell. As the virus approaches the cell (*1*), a viral protein designated gp120 binds to a cell-surface molecule known as CD4 (*2*). That interaction uncovers another protein called gp41. One end of the gp41 molecule embeds itself in the cell membrane (*3*), leading to the eventual fusion of the viral membrane and the cell membrane (*4*).

presence of the HIV receptor. Even VSV(HIV) pseudotypes were unable to infect them, although VSV, once it enters mouse cells, can usually replicate perfectly well. These results suggest another component of the cell surface is required for the virus to achieve full entry after it has bound to the cell membrane. The nature of this second factor is not known.

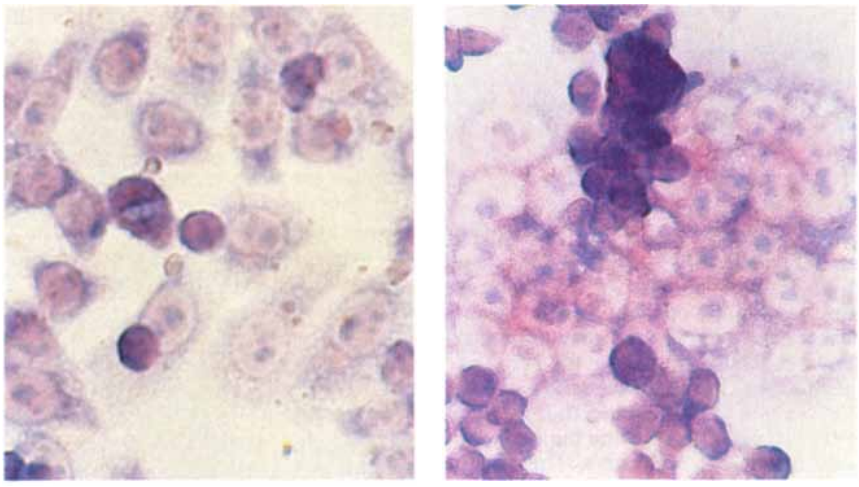
The binding of viral gp120 to cellular CD4 is only the first step of viral entry into the cell. The later steps have been less thoroughly elucidated. For example, how does the virus's genetic material enter the cell? The simplest and likeliest possibility is that the viral membrane simply fuses with the cell membrane, injecting the core of the virus (including its genetic material) into the cell. Another possibility is that the cell membrane forms a small pocket that later becomes an enclosed sac called an endocytic vesicle. The vesicle completely surrounds the virus particle and carries it into the cell. Then a reaction within the cell acidifies the membrane of which the vesicle (now called an endosome) is made. When the endosome is acidified, it undergoes a conformational change and fuses with the viral membrane, releasing the viral core into the cell's interior.

Recent evidence casts doubt on the relevance of this mechanism, which is known as receptor-mediated endocytosis. Barry S. Stein of the Stanford University School of Medicine and Myra O. McClure of our laboratory have shown independently that the entry of HIV into the cell is independent of acidity: drugs that block the acidification of endosomes do not prevent HIV infection. In addition, Dan R. Littman of San Francisco and Maddon have shown that mutations in the "tail" of the CD4 antigen (the part within the cell) that prevent the antigen's incorporation into endosomes do not inhibit HIV infection. It is likely, then, that HIV enters the cell by fusing directly with the cell membrane.

The direct-fusion mechanism would also help to explain the cell-to-cell fusion that leads to the formation of syncytia. Syncytia form because HIV-infected cells manufacture gp120 and carry it on their cell membrane. When an infected cell meets a healthy cell that bears the CD4 antigen, the gp120 of the infected cell can bind to the CD4 of the healthy cell. Then the two cells join, probably by direct fusion. The resulting syncytium continues to carry gp120 on its cell membrane, and so it can continue to fuse with healthy



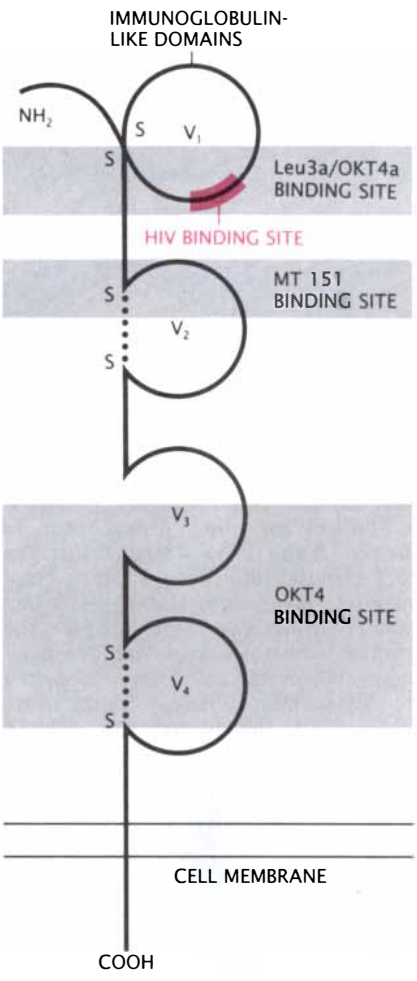
ENTRY of the virus's core, including its genetic material, into the target cell probably takes place by one of two mechanisms. The likeliest (*top*) is direct fusion. In this mechanism the virus particle binds to the cell (*1*) and the viral membrane fuses with the cell membrane (*2*), ejecting the core material into the cell (*3*). The other mechanism (*bottom*), called receptor-mediated endocytosis, also begins when the virus particle binds to the cell membrane (*1*). In the next stage, however, the cell membrane buckles inward to form a pocket (*2*) known as a coated pit. The membrane encloses the virus particle (*3*) and detaches from the cell surface to form a body called an endosome (*4*). Eventually the viral membrane fuses with the membrane of the endosome (*5*), releasing the viral core into the interior of the cell (*6*).



MULTINUCLEATED SYNCYTIA, clusters of many nuclei within a single cell membrane, are a sign of HIV infection in cell cultures. They form when infected cells, which make gp120 and carry it on their surface, fuse with healthy cells bearing the CD4 molecule. The photograph at the left shows HeLa cells, a line of cervical-cancer cells that do not make the CD4 molecule and cannot be infected with HIV. They have been exposed to HIV, but no syncytia have formed. The photograph at the right shows HeLa cells that have been genetically altered so that they make the CD4 molecule. These cells, on being exposed to HIV, have become infected and have formed syncytia.

cells. One infected cell may eventually bring together as many as 50 cells.

In any case, whether direct fusion or receptor-mediated endocytosis is the correct model, the viral membrane must fuse with a membrane of the cell. How does that happen? According to a plausible model, the binding of gp120 to CD4 causes a change in the shape of the gp120 protein, revealing a part of another envelope protein, known as gp41, that is normally hidden under the gp120 molecule. This region of gp41 is hydrophobic: it will embed itself in a cell membrane rather than remaining exposed to the aqueous solution surrounding the cell. Once it is uncovered, the hydrophobic region of gp41 interacts with the adjacent cell membrane and induces the viral membrane and the cell membrane to fuse together. It is not clear whether some receptor on the cell surface other than the CD4 antigen binds to gp41 or whether gp41 embeds itself directly in the cell membrane.



CD4 MOLECULE cannot yet be depicted in detail, but some features of its structure are known. Most of the molecule lies outside the cell, but a segment of it passes through the cell membrane and ends in a short tail inside the cell. Four sections of the molecule, designated V1, V2, V3 and V4, resemble the so-called variable domains of some immunoglobulin (antibody) molecules. The site to which the HIV gp120 molecule binds (*color*) lies in the outermost section. Shaded regions indicate areas in which binding sites of certain monoclonal antibodies (antibodies that recognize specific molecular configurations) lie. The so-called Leu3a/OKT4a group of monoclonal antibodies binds at the same site as HIV and can block infection by HIV.

After HIV enters the cell, its genetic material, which is encoded in RNA, is converted into DNA. The DNA "provirus" is then integrated into the DNA of the target cell. This means that the infection is persistent for the cell's lifetime and that of its progeny if it multiplies. The integrated virus may remain completely "silent," or else it may manifest itself in any one of at least three ways.

First, the viral genome may cause a persistent infection, in which some new virus particles are created but few cells are killed. Second, infection may lead to the creation of syncytia, which die soon after forming. Syncytia are a dominant effect of HIV infection in cell culture. In human beings they are sometimes seen (particularly in the brain) during later stages of infection, but it is not clear whether they play a role in the early pathogenesis of AIDS.

A third possible result of HIV infection is the rapid death of cells without the formation of syncytia. It is not yet known how HIV kills cells. Perhaps some product encoded by the HIV genes is directly toxic. Alternatively, perhaps the gp120 that is made and embedded in cell membranes as a result of infection binds to CD4 that is already there; such binding could damage the cell's membrane systems. The host's immune response also shapes the fate of infected cells, since the immune system can recognize viral proteins on the surface of infected cells and destroy them.

The distribution of HIV-infected cells in the body is determined primarily by the distribution of cells bearing CD4. The CD4 antigen was first identified by its presence on certain *T* cells, and indeed much of its normal function seems to involve assisting the complex network of communication among immune cells.

T cells bearing CD4 interact with cells known as antigen-presenting cells, which locate foreign antigens and display them on their own cell membrane, together with molecules known as Class II Major Histocompatibility Complex (MHC) glycoproteins. When helper *T* cells recognize this combination of an antigen and a Class II MHC glycoprotein, they initiate an immune response against other cells bearing the antigen, such as foreign or infected cells. It is thought that an interaction between CD4 antigens on the *T* cells and Class II MHC glycoproteins on the antigen-presenting cells is a crucial part of the encounter between the cells.

It is now known that *T* cells are not the only cells that have the CD4 antigen embedded in their membrane. As many as 40 percent of the peripheral-blood monocytes (cells that mature to become the scavenger cells known as macrophages), as well as certain antigen-presenting cells in the lymph nodes, skin and other organs, also express CD4 and can be infected by HIV. About 5 percent of the body's *B* cells (cells responsible for the production of antibodies) may also express CD4 and be susceptible to infection by HIV. In all these cells the presence of CD4 can be shown relatively easily.

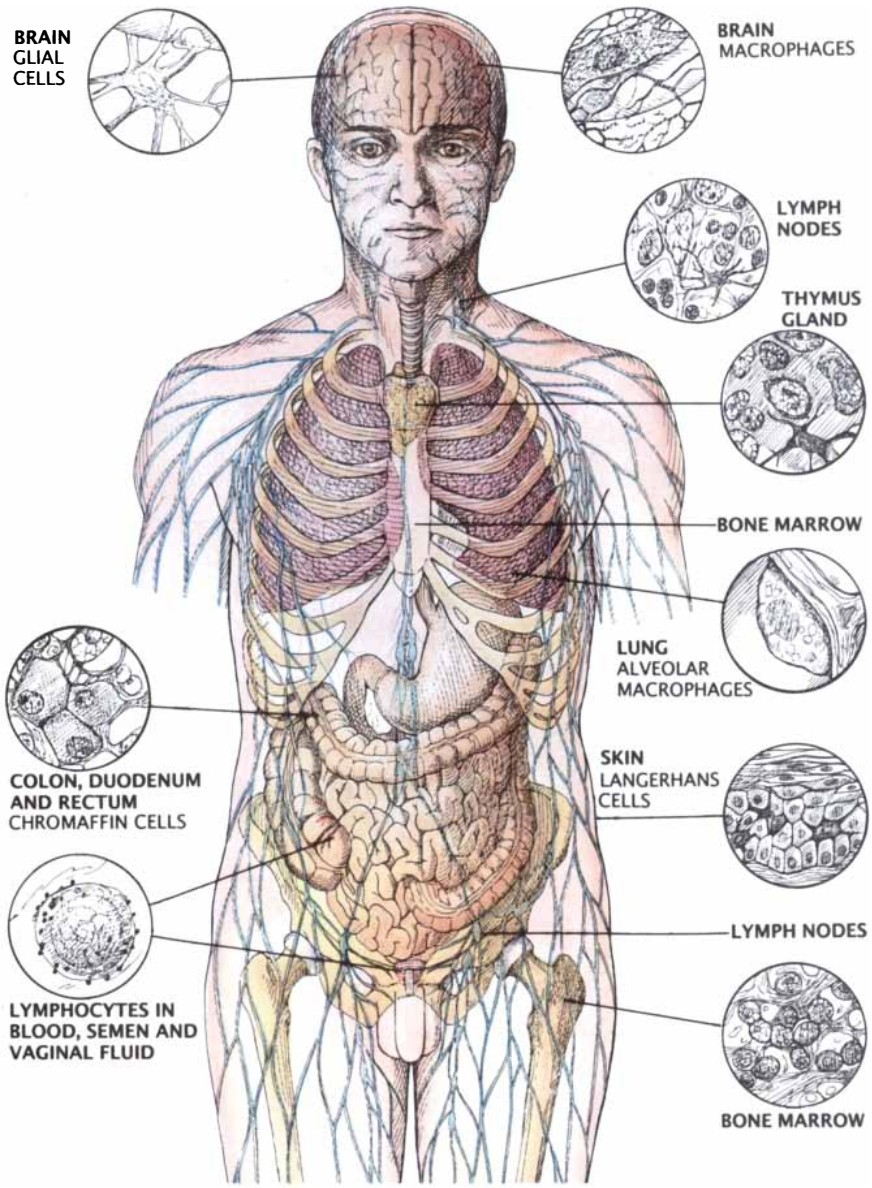
On the other hand, in some other kinds of cells that can be infected by HIV in culture it is not possible to detect CD4 directly. These include certain cells of the brain known as glial cells, a range of malignant brain-tumor cells and some cell lines derived from cancers of the bowel. Nevertheless, although these cells do not produce detectable amounts of CD4, they do contain low levels of messenger RNA encoding the CD4 protein, indicating that they produce some CD4. Apparently the expression of only a very small amount of CD4 is sufficient for infection by HIV.

Cells of the gut also do not produce appreciable amounts of CD4, but Cecilia Cheng-Mayer and Levy at San Francisco have recently shown that the gut cells known as chromaffin cells do sometimes appear to be infected with HIV in vivo. They suggest that such a gut infection may be what leads to the AIDS-associated weight loss and emaciation known in Africa as Slim Disease. The role of CD4 in infections of brain cells and gut cells in vivo cannot be determined without further research. It is possible that in these cases the HIV particle binds to an alternative receptor molecule.

A number of workers have recently determined precisely which part of the CD4 molecule is the binding site for HIV. Most of the molecule lies outside the cell, but a small

segment passes through the cell membrane and ends in a short intracellular "tail." The extracellular region consists of four domains that are similar in some ways to the "variable domains" of antibody molecules.

One way to determine the precise location of the binding site is to expose CD4 molecules to monoclonal antibodies that recognize various epitopes, or molecular shapes, on the CD4 molecule and note which antibodies block the binding of HIV to CD4. One group of antibodies, represented by the antibodies designated Leu3a and OKT4a, is particularly efficient at blocking the binding of HIV. Quentin J. Sattentau and Peter C. L. Beverley of University College London have used large panels of anti-CD4 monoclonal antibodies to draw a "map" of the HIV binding site (that is, to determine which regions of the CD4 molecule are most important in binding HIV). They have found that the Leu3a antibody blocks not only HIV-1 and HIV-2 but also many strains of the simian immunodeficiency virus (SIV) [see "The Origins of the AIDS Virus," by Max Essex and Phyllis J. Kanki, page 64]. One implication of this finding is that the region of gp120 that is most important in binding to the cell is highly conserved, even among strains of virus whose envelope proteins are otherwise very different, having fewer than 40 percent of their amino acids (the basic building blocks of protein) in common. In another study, McClure and Sattentau have examined how well various epitopes of CD4 have been conserved during the course of evolution. They have demonstrated that the Leu3a monoclonal antibody reacts with all primate lymphocytes, including those of human beings, the great apes and African, Asian and New World monkeys, and prevents them from being infected in vitro with HIV. (In vivo most monkeys are not susceptible to HIV infection.) The implication is that the relevant parts of CD4 have been preserved even as the ancestors of these species diverged in other ways.



DISTRIBUTION OF TISSUES in the body that can be infected with HIV is closely linked to the distribution of cells bearing the CD4 molecule. With the possible exceptions of glial cells in the brain and chromaffin cells in the colon, duodenum and rectum, every cell that can be infected with HIV carries the CD4 molecule on its surface.

A further way to determine which parts of the CD4 and gp120 molecules are crucial for binding is to introduce deliberate mutations in the genes that encode the molecules. For example, an investigator might simply delete the genetic sequence that encodes a region of the CD4 molecule and test the resulting mutant's ability to bind HIV.

Early experiments, in which large sections of the CD4 molecule were deleted, indicated that the amino-terminal domain of the molecule (the section farthest from the cell membrane) is essential for the binding of gp120. Ned Landau and Littman at San Francisco have confirmed these results in experiments in which segments of mouse CD4 were combined with segments of human CD4. Mouse CD4 is broadly similar to the human molecule, but it is not recognized by gp120 or by the monoclonal antibodies that are specific to human CD4. The "chimeric" molecules do bind gp120 very well if the first 100 amino acids at the amino-terminal end of the molecule are human, even if the rest of the molecule is derived from the mouse. (The CD4 molecule as a whole consists of 433 amino acids.)

In experiments that were even more specific, Andrew Peterson and Brian Seed of the Harvard Medical School made hundreds of tiny "point mutations" in the human CD4 gene. They found that about seven amino acids residing near the middle of the initial 100-amino-acid segment are crucial for recognition by gp120 and by such monoclonal antibodies as Leu3a and OKT4a, which can block the binding of gp120. The major site on CD4 that is recognized by gp120, then, is a small region in the outermost part of the CD4 molecule.

The parts of gp120 that are essential for binding have also been analyzed by mutagenesis. William A. Haseltine's

group at the Dana-Farber Cancer Institute and Larry Lasky's group at Genentech Inc. have shown that three distinct regions of gp120 are essential for the recognition and binding of CD4. Probably these regions come together to form a pocket that fits the binding site on CD4 when the gp120 molecule folds into its normal three-dimensional configuration.

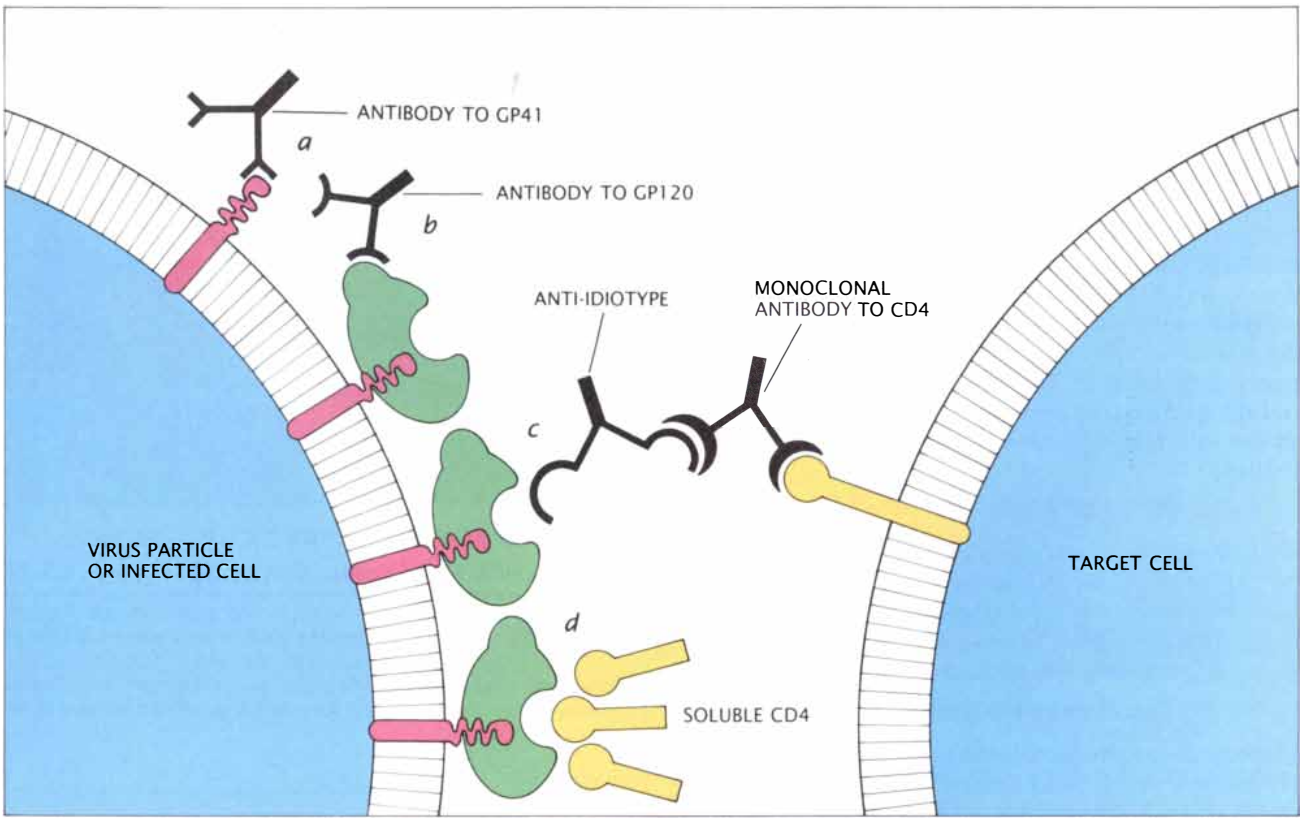
Knowledge of the interactions through which HIV binds to target cells suggests several possible ways of blocking HIV infection. One method would be to inject subjects with so-called soluble CD4 molecules, which consist of segments of the portion of CD4 that normally lies outside the cell membrane. Soluble CD4 has been produced through recombinant-DNA technology by a number of laboratories and biotechnology companies. The molecules bind tightly to gp120; when they saturate all the gp120 on the virus's envelope, they neutralize its infectivity. Because the CD4-binding site on gp120 is essentially the same in all strains of HIV and SIV, soluble CD4 can neutralize any strain of the virus, making it an attractive candidate for treatment.

Soluble CD4 would have some disadvantages as an AIDS therapy, however. First of all, it would have to be injected repeatedly in large doses. In addition, soluble CD4 might bind to Class II MHC glycoproteins, interfering with their normal function. That would exacerbate the immune deficiency of AIDS rather than curing it. The problem could be surmounted, however, if gp120 and the Class II MHC glycoproteins recognize different sites on CD4. It might then be possible to make smaller segments of the CD4 molecule that correspond just to the site recognized by gp120.

Another way to exploit our knowledge of the CD4 molecule involves molecules known as anti-idiotype antibodies [see "Anti-idiotypes and Immunity," by Ronald C. Kennedy, Joseph L. Melnick and Gordon R. Dreesman; SCIENTIFIC AMERICAN, July, 1986]. A number of investigators, led by Ronald C. Kennedy and Gordon R. Dreesman of the Southwest Foundation for Biomedical Research in San Antonio, have inoculated mice with monoclonal antibodies that recognize the part of CD4 that is the binding site for gp120. These monoclonal antibodies are, in a sense, "negative images" of the binding site: they fit around the binding site on CD4 as a mitten fits a hand. In response to such an inoculation, the mouse immune system generates antibodies that bind to the monoclonal antibody. Some of these new antibodies, the so-called anti-idiotypes, fit precisely into the monoclonal antibody's CD4-binding site; they are new hands that fit inside the mitten.

In some cases the anti-idiotype has a shape very similar to that of the site on CD4 that is recognized by gp120. In a sense, then, these anti-idiotypes resemble CD4; like soluble CD4, they can bind to viral gp120 and should therefore be able to neutralize the infectivity of HIV.

Thus it may be possible to use anti-CD4 monoclonal antibodies as a kind of vaccine in human beings. In response to an injection of anti-CD4 monoclonal antibodies, the immune



POTENTIAL AIDS THERAPIES might block the binding of the virus particle or an infected cell to a target cell. Among the simplest therapies are antibodies that bind to gp41 (*a*) or to gp120 (*b*). In another approach (*c*) the subject would be inoculated with monoclonal antibodies that bind to the CD4 molecule. The presence of these antibodies might stimulate the patient's immune system to produce "anti-idiotypes": a second set of antibodies, which would bear some resemblance to the CD4 molecule. The anti-idiotypes might bind to gp120 molecules, blocking them off and preventing them from binding to CD4 on target cells. Still another approach (*d*) would be to inject the subject with "soluble CD4" molecules (which consist of the portion of CD4 that normally lies outside the cell membrane). Soluble CD4 would bind tightly to gp120, blocking infection.

system might produce anti-idiotypes that bind to the virus and neutralize it. These anti-idiotypes would protect against all strains of the virus, because all strains of HIV recognize the same site on the CD4 molecule.

The actual neutralizing effect of such anti-idiotypes has been investigated independently by Dalgleish and by Sattentau and Beverley. They find that anti-idiotype antibodies do indeed neutralize HIV, but only very weakly. There are several possible explanations for such weak neutralization. First, it may be that the anti-idiotype antibody does not fit the gp120 protein very well. Second, the part of the gp120 molecule that recognizes CD4 probably resides in a pocket or crevice within the molecule, so that the relatively large antibodies cannot gain access to it easily. Third, the anti-idiotypes may not actually neutralize HIV at all; instead they may stimulate the immune system to produce another set of antibodies that have the opposite affinity: anti-anti-idiotypes, which might block the CD4 receptor just as the original antibody does.

People who are infected with HIV generate an impressive immune response to the virus. They produce antibodies to all the viral proteins, and their immune systems activate the various types of killer and scavenger cells that are part of any normal immune response. Yet once infection has occurred, these responses do not appear to halt the progress of the disease. Perhaps our increasing knowledge of the viral envelope and the cellular protein to which it binds will provide new approaches to defeating the virus.

FURTHER READING

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