The Immune System in AIDS

The AIDS virus alters the growth and function of T4 lymphocytes, a class of white blood cells that is crucial to the immune system. New knowledge of how the virus does so may lead to treatments and perhaps a vaccine

by Jeffrey Laurence

In 1981 the Centers for Disease Control in Atlanta recognized the first cases of a fatal new disorder that came to be known as acquired immune deficiency syndrome (AIDS). Its victims died of a variety of rare infections and malignancies, among them a pneumonia caused by the protozoan *Pneumocystis carinii* and Kaposi's sarcoma, a cancer of the lining of blood vessels. They also suffered from other "opportunistic" infections, caused by microorganisms that are ubiquitous but ordinarily not able to cause disease. Indeed, the infections and cancers seen in AIDS patients were previously known only in people born with certain defects in their immune system and in patients whose immunity had been impaired by cancer chemotherapy or the immunosuppressive drugs given for organ transplantation. AIDS, it appeared, killed its victims by destroying their immune system.

Since the disease was first recognized the number of cases has risen swiftly; in the U.S. alone the figure reached 14,000 by late 1985. Knowledge of AIDS has increased proportionately. Françoise Barré-Sinoussi, Jean-Claude Chermann and Luc Montagnier at the Pasteur Institute in Paris and a group led by Robert C. Gallo at the National Cancer Institute independently identified the causative agent, a virus of the retrovirus family, in 1983 and 1984 respectively; the French group called it LAV (lymphadenopathy-associated virus) and the American workers HTLV-III (human *T*-lymphotropic virus type III).

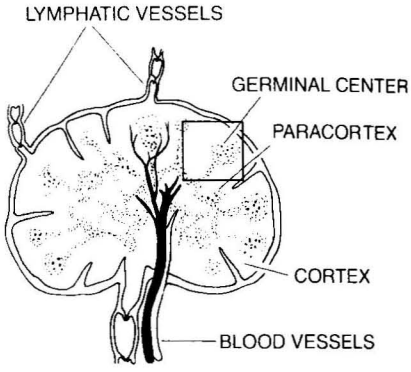
The groups at highest risk for infection have become increasingly well defined; they include homosexual and bisexual men, abusers of injected drugs, the sexual partners of people in AIDS risk groups, and children born to mothers at risk. Recipients of blood transfusions and blood products have also contracted AIDS, but the screening of donated blood for evidence of infection has drastically reduced their risk. The fact that the disease shows no sign of spreading beyond those groups, except to predictable targets such as women who are artificially inseminated with sperm from infected donors, indicates that the virus is ordinarily transmitted only through the blood or through sexual intercourse. All epidemiologic evidence indicates that food, water, insects and casual contact do not spread AIDS.

An understanding of the mechanisms by which the disease cripples the immune system is also emerging. The knowledge may make it possible to lessen the effects of the disease, and eventually to prevent and cure it. It also underscores the intricacy of the immune system. It now appears that the total collapse of the immune defenses in AIDS victims stems largely from a single defect: a reduction in the number and a change in the function of the *T*4 lymphocytes, one of the many distinct kinds of cells that make up the immune system.

The Immune System

How is it that by damaging a single link the AIDS virus causes the immune system as a whole to unravel? The answer lies in the complex web of interactions among the different classes of blood cells that take part in immunity. The immune system is a flexible but highly specific defense mechanism that kills microorganisms and the cells they infect, destroys malignant cells and removes debris. It distinguishes such threats from normal tissue by recognizing antigens, or foreign molecules, and mounting a response that varies with the nature of the antigen.

Over a lifetime many thousands of different antigens challenge the body. The cells of the immune system can recognize and respond to virtually every antigen because they are divided into millions of clones, each of them made up of one or more cells specialized to recognize one or a few distinct antigens. In addition to this differentiation by antigen specificity, the cells also diverge into several types as they mature from common precursor cells. The cell types can be distinguished by the contexts in which they recognize antigen, by the molecular characteristics of their surface membranes and



LYMPH-NODE DISRUPTION is characteristic of AIDS and the collection of symptoms, called AIDS-related complex (ARC), that is one of its precursors. Fluorescence micrographs of sections of lymph node show the structures outlined in the diagram (*above*): a germinal center and some of the surrounding cortex and paracortex. In a normal node (*top*) the white blood cells known as *T*8 lymphocytes (*orange*) populate the paracortex but are not present in the germinal center, in which *B* lymphocytes and the structural cells of the node, the dendritic reticular cells (*green*), form a regular network. A section of a swollen node from a patient with ARC (*bottom*) contains many more *T*8 cells; they invade the germinal center, whose regular structure is disrupted. It is not known how the AIDS virus causes these changes in node architecture, but they may have a relation to the immune deficiency: the surface membranes of normal dendritic reticular cells are thought to trap and retain foreign proteins that trigger an immune response. Lymph-node disruption may also serve as an early clue to infection with AIDS virus. George Janossy of the Royal Free Hospital in Hampstead, England, made the images.

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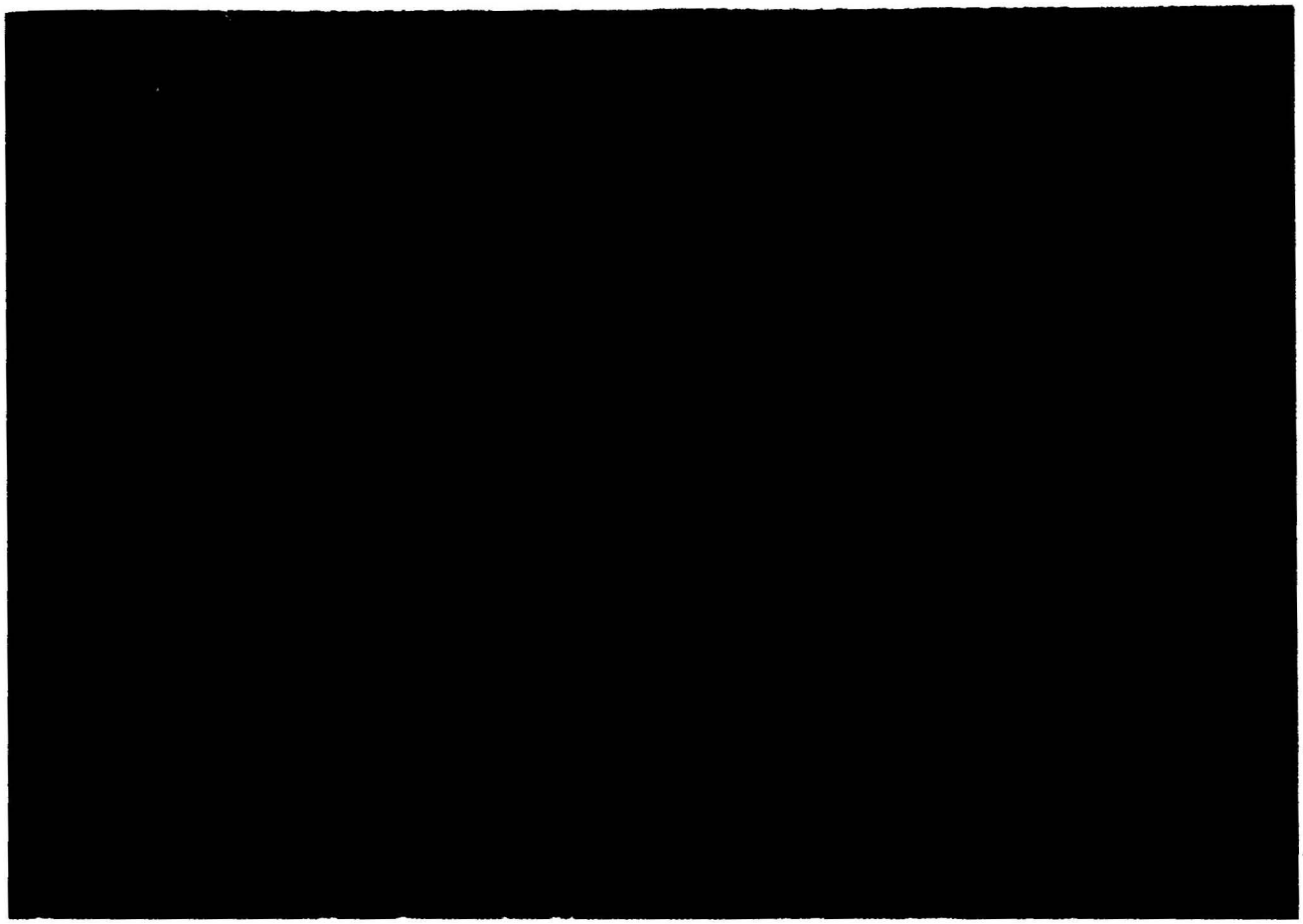

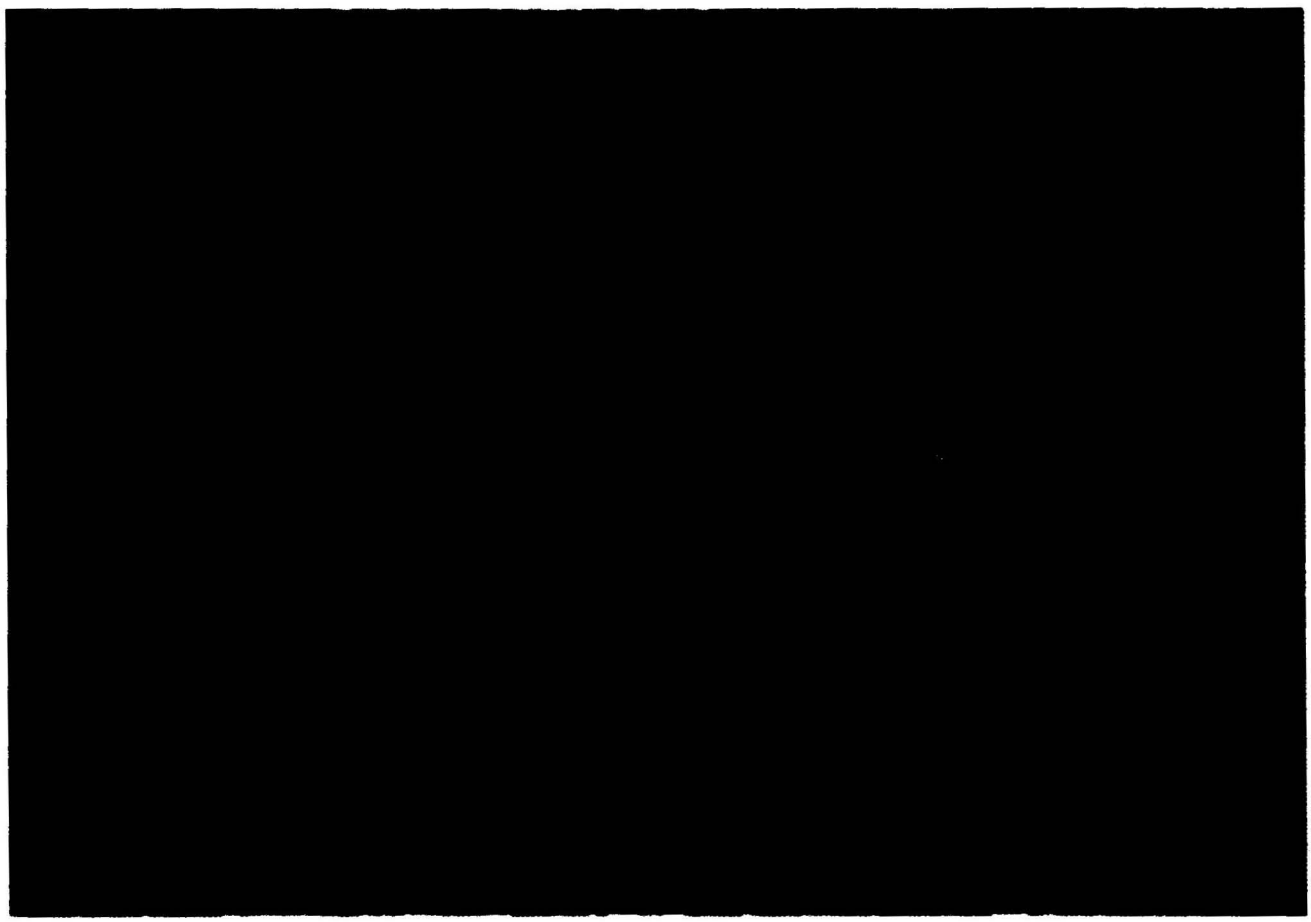

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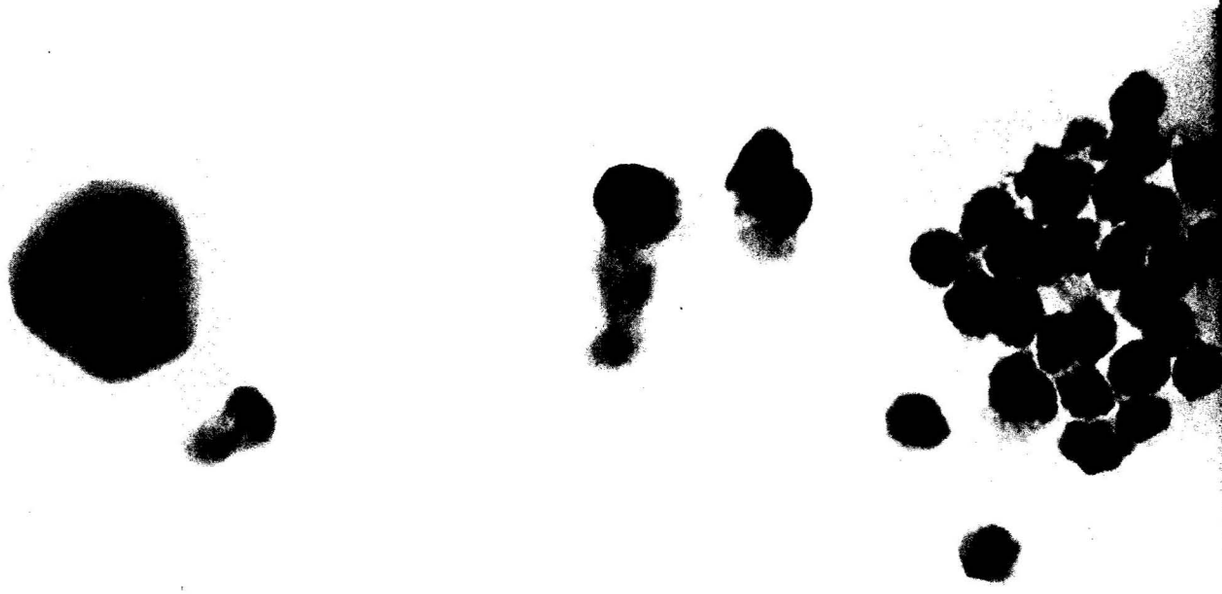
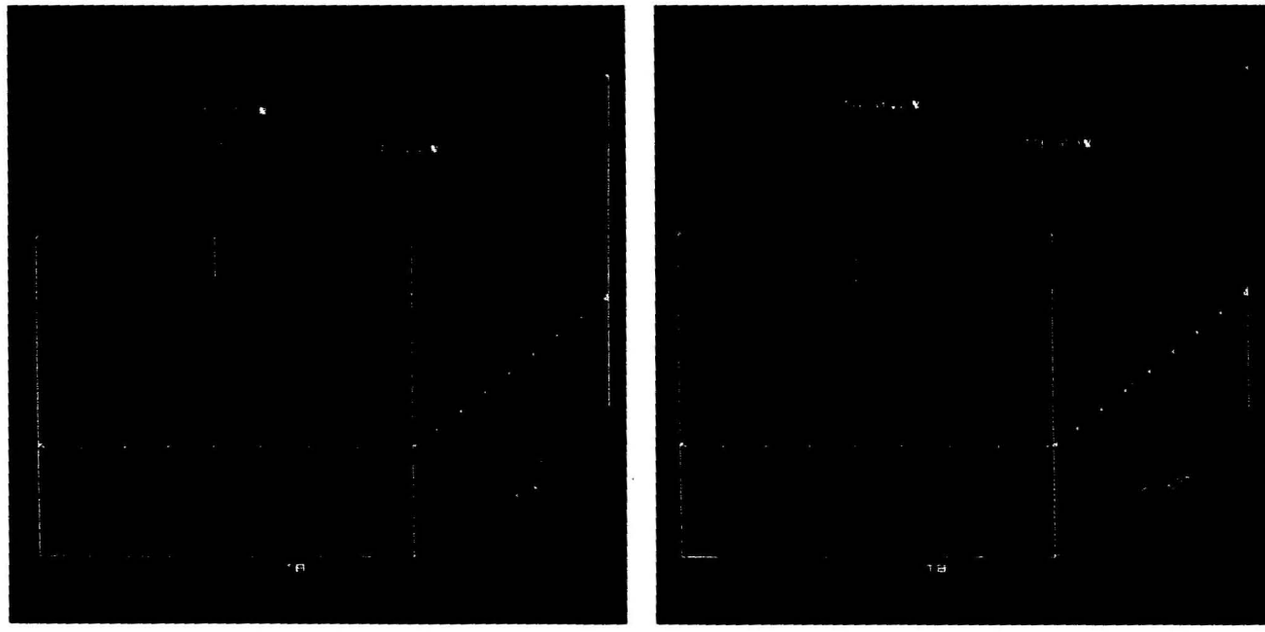
...the *B* cells, so named because they develop in the bone marrow, and the *T* cells, which originate in the bone marrow but complete their ... removal or destruction. When a *B* cell recognizes an antigen, which may be circulating in the blood or the lymph or displayed on the surface of an infected cell, it becomes activat... molecules on their membrane that ... as specific receptors for the antig... Some of the cells, known as plas... cells, actively secrete antibody. Oth... longer-lived *B* cells are one root of ... immunity that forestalls recurrence...



INFECTED *T* CELLS (*left*) contrast with normal *T* cells (*right*) because the AIDS virus causes the membranes of infected cells to fuse, yielding multinucleated complexes known as syncytia. The virus primarily infects *T4* cells, which look like other *T* cells but differ from them in function and biochemical markers. Fusion of infected cells with normal ones might spread virus to other tissues.



RATIO OF *T*-CELL TYPES is inverted in AIDS. In normal blood the ratio of *T4* cells (*blue*), which induce the proliferation of lymphocytes and help other cells to mount an immune defense, and *T8* cells (*yellow*), which destroy infected tissue and shut down the immune response, is about two to one (*left*). In the blood of AIDS patients a drastic decrease in the number of *T4* cells leaves *T8* cells as the predominant type (*right*). The computer-generated graphs show results from fluorescence-activated cell sorting, a technique in which antibodies specific for cell-surface proteins are used to label the *T4* and *T8* molecules with dyes that fluoresce in different colors. The cells then pass through automated equipment that counts and separates them. In the lower graphs the vertical axis corresponds to the number of cells and the horizontal axis to fluorescence intensity. The middle representations combine the graphs, plotting cell number against fluorescence intensity for both dyes at once. Unlabeled peaks represent cells bearing neither *T4* nor *T8* markers.

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The T cells are more complex in classification and function. Most T cells cannot recognize free antigen circulating in the blood or the lymph, and they can respond to antigen on a cell surface only under particular conditions. The foreign substance must be displayed in conjunction with one of the host cell's own proteins: molecules coded for by the segments of DNA making up the Major Histocompatibility Complex (MHC). For an immune response to ensue the antigen receptor on the surface of a T cell must simultaneously recognize the antigen and the MHC protein.

Once a T lymphocyte has recognized antigen it performs a function that depends on its subclass. Only one kind of T cell actively defends the body: the cytotoxic T cells, which destroy infected, foreign or malignant cells by lysing them (disrupting their cell membrane). The other kinds of T cells modulate the immune response by secreting messenger proteins or by direct contact with the participating cells.

Inducer T cells trigger the maturation of T lymphocytes from precursor forms into functionally distinct cells. Helper T cells are a precondition for the action of other T cells and most B cells; having recognized a specific antigen, they enable cytotoxic T lymphocytes to destroy cells bearing the antigen and B lymphocytes to secrete appropriate antibody. The fourth kind of T cells, suppressor T cells, dampen the immune response of B and T cells, in effect shutting down the immune defenses several weeks after an infection activates them. Helper and suppressor T cells interact in complex ways: they have opposite effects on cytotoxic cells, and in shutting down the immune response suppressor cells also turn off helper cells.

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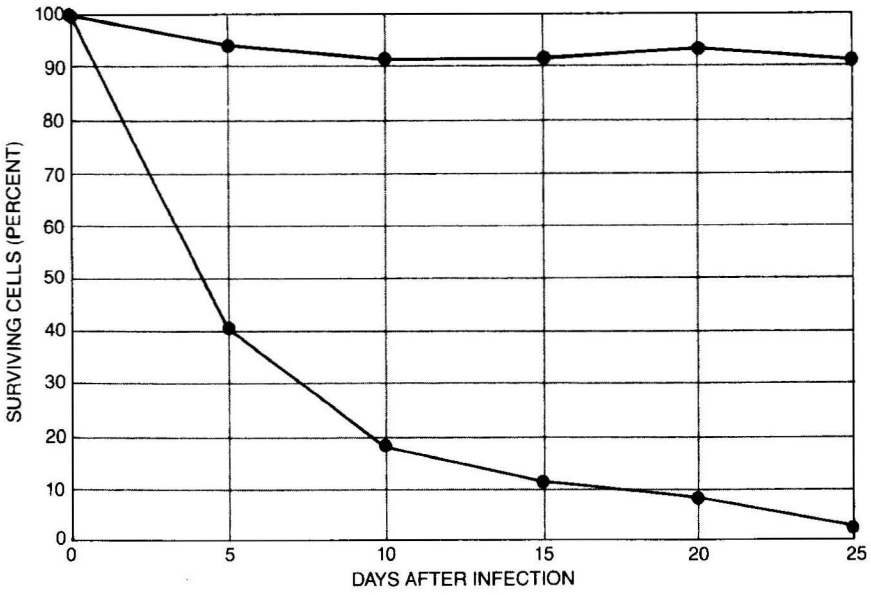
$T4$ and $T8$

The T cells thus make up four subsets on the basis of function, but on the basis of biochemical markers on their surface there are two main kinds: $T4$ cells, which have helper and inducer roles, and $T8$ cells, with suppressor and cytotoxic functions. $T4$ and $T8$ cells are also set apart by the kind of MHC protein that must be associated with an antigen if they are to recognize it. $T8$ cells recognize antigen in the context of Class I MHC proteins, a kind of molecule that is present on the surface of all nucleated cells. Hence a cytotoxic $T8$ cell ordinarily can kill any infected cell that carries an antigen for which the $T8$ cell is specific. $T4$ cells, in contrast, respond to antigen that is associated with Class II MHC proteins, which are found primarily on the surface of specialized cells known as antigen-presenting cells.



SURVIVAL OF T CELLS in culture after exposure to the AIDS virus differs by cell type. The virus has little effect on the number of $T8$ cells (*black*). It causes the number of $T4$ cells, its preferred host, to decline dramatically (*color*). The virus affects the replication of infected cells. It also prevents infected cells from displaying the $T4$ marker protein on their surface membrane, and a protein from the viral envelope binds to and masks the $T4$ marker when it is displayed. The last two effects compound the measured decline of $T4$ cells.

Antigen-presenting cells and natural killer cells are the other major actors in the immune response. Chief among the former are the macrophages (scavenger cells that develop from monocytes, a kind of white blood cell) found in the skin and other tissues. The Langerhans cells of the skin and the dendritic cells of the blood, lymph nodes and spleen also present antigen. Macrophages function by engulfing a virus or other intruder, enzymatically breaking down its proteins in a highly specific way and displaying the antigenic protein fragments on the cell membrane together with Class II MHC proteins. Macrophages thereby prepare the antigen for recognition by $T4$ cells. Natural killer cells, a peripheral arm of the immune system, kill virus-infected cells and tumor cells spontaneously, without directly interacting with lymphocytes or recognizing antigens.

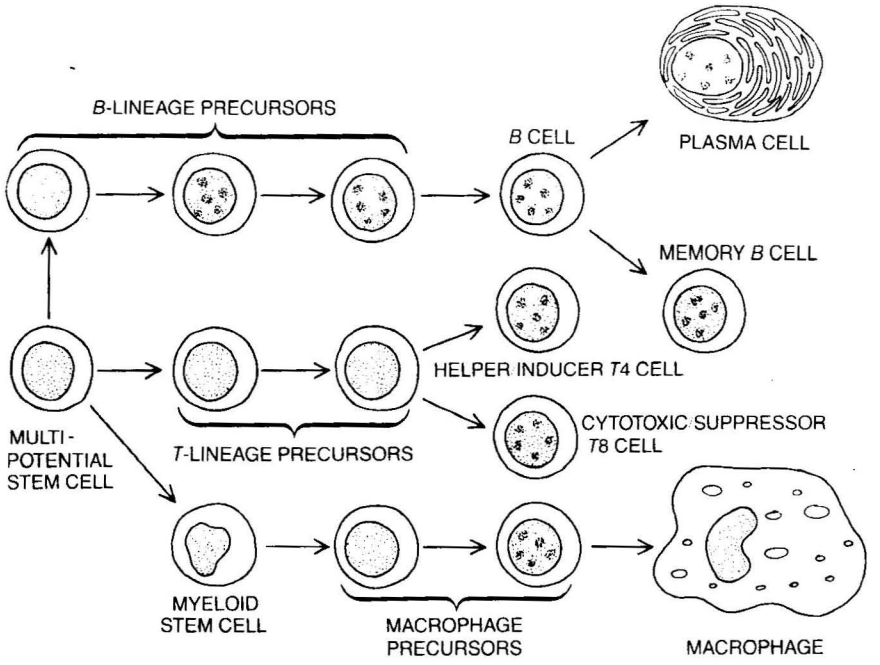
Responses to Infection

An invading virus ordinarily evokes a complex interplay of these cellular elements. To appreciate the intricacy of the phenomenon, it is worth tracing the immune response to an ordinary viral infection such as measles. Virus-infected cells secrete proteins known as interferons, which stimulate a first line of defense against the measles infection: natural killer cells. Their activity peaks within a day or two after infection. The next combatants are the macrophages, which engulf and degrade the virus. They thereby set in motion the T-cell response.

The macrophages stimulate the immune response not only by processing the viral antigens into a form that helper and inducer T cells can recognize but also by secreting soluble proteins known as monokines. One of the monokines secreted by certain macrophages is gamma-interferon; another is interleukin-1. Interleukin-1 has the ability to activate T cells that have recognized the viral antigen on a cell surface, preparing them to differentiate and divide. Together the interleukin-1 and gamma-interferon cause the fever and malaise that accompany measles and most other viral infections.

Activated T cells themselves produce soluble factors, which are known as lymphokines. It is a lymphokine called interleukin-2, secreted by $T4$ and $T8$ cells, that mediates the next step in the immune response. Under the influence of interleukin-2, T cells that have been stimulated by antigen and interleukin-1 proliferate into enlarged clones of mature cells: cytotoxic, suppressor and helper T cells. In a response that peaks about a week after infection, the cytotoxic T cells lyse measles-infected cells; the elimination of infected cells at this stage can bring the infection to a rapid halt.

The suppressor cells, whose number



CELLULAR PARTICIPANTS in the immune defense diverge from common precursors: the multipotential stem cells found in bone marrow. *B* cells develop through various stages in the bone marrow and then are released into the bloodstream or lymph. Contact with antigen stimulates them to differentiate into plasma cells, which secrete antibody, and memory cells, which are responsible for lasting immunity. The precursors of *T* cells migrate to the thymus gland to mature. After their release into the blood or lymph *T* cells take on distinct biochemical and functional identities as *T*4 cells, with helper and inducer roles, and *T*8 cells, with cytotoxic and suppressor roles. Macrophages begin developing in the bone marrow as myeloid stem cells, which also give rise to several other classes of white blood cells (not shown). Macrophage precursors circulate in the blood; mature macrophages migrate to the skin, the spleen and the lymph nodes, where they are most likely to encounter antigen.

increases more slowly than that of cytotoxic cells, help to shut down the *T*-cell response after several weeks. Following suppression a population of memory *T* cells persists, probably for life. Memory *T* cells, like memory *B* cells, mediate recall reactions: accelerated responses to subsequent encounters with the antigen.

Central to this *T*-cell activity are the helper and inducer *T*4 cells. Without their influence, exerted through lymphokines or through direct contact, neither the cytotoxic nor the suppressor cells could function. *T*4 cells also influence other aspects of the immune defense. The interleukin-2 they secrete bolsters the natural killer cells, and they produce gamma-interferon (considered a lymphokine when it is secreted by *T* cells), which stimulates macrophages in their role of engulfing virus and presenting antigen.

*T*4 cells are also vital to the production of antibody, the second and less important phase of the body's initial defense against a viral infection such as measles. (Antibody plays a larger role in the phenomenon of lasting immunity.) To respond to the measles antigen the *B* cells require a signal from the helper *T* cells, in the form either of lymphokines or of direct contact. The antigen-specific *B* cells then multiply into an enlarged clone of antibody-secreting plasma cells and a population of memory *B* cells. The plasma cells secrete two main classes of antibodies to the measles virus; their levels peak respectively one week and three weeks after infection, before suppressor *T*8 cells (also aided by *T*4 cells) dampen the *B*-cell response.

Foiling Immunity

Just as surely as these mechanisms defeat measles, they would block infection with the AIDS virus if it did not have a counterstrategy. The AIDS virus is not unique in its ability to avoid destruction by the immune system. Certain viruses, such as the agent of caprine arthritis encephalitis (CAE), a degenerative disease of goats, do so by triggering an inappropriate immune response, for example by eliciting the production of antibodies that do not block the infection.

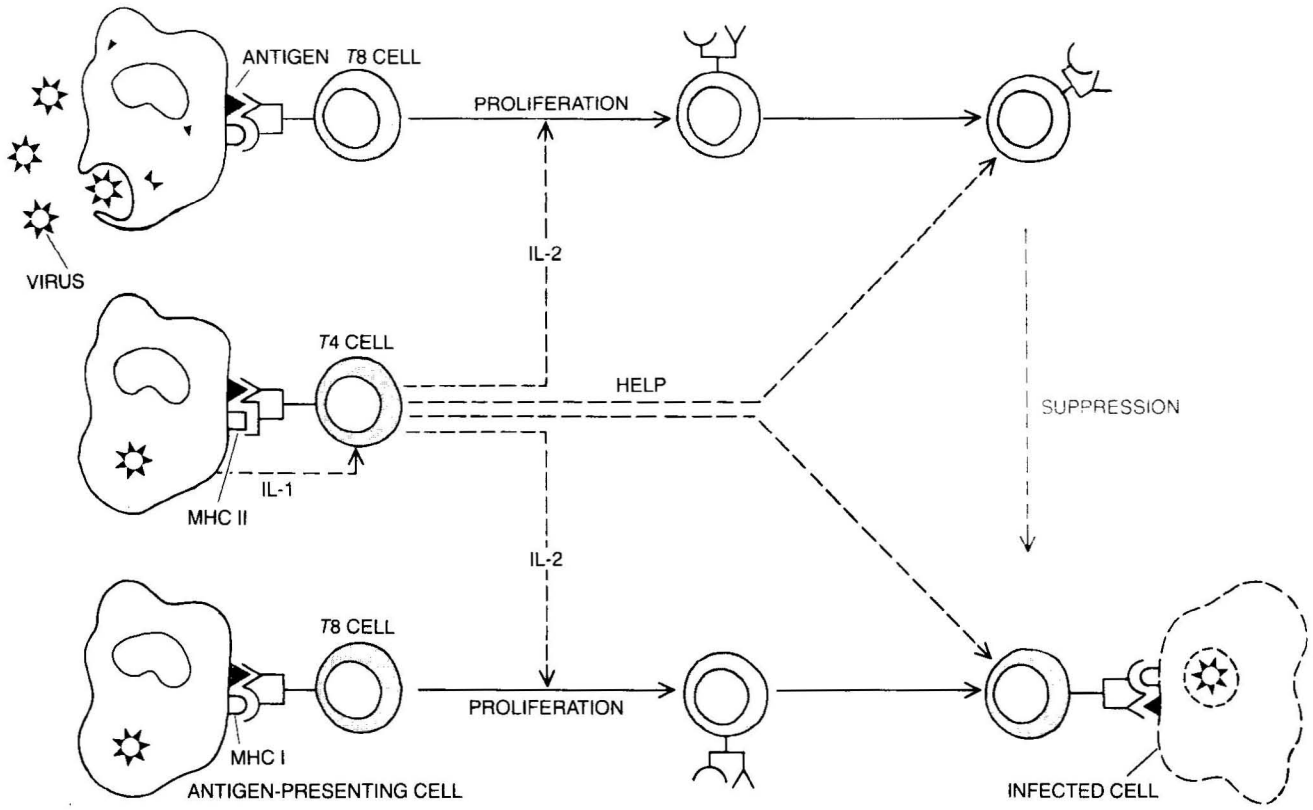
A virus could also evade the immune system if its proteins changed frequently because of mutations, causing any antigen-specific immune response to miss its mark. Sheep with a disease known as visna at first produce antibodies that can counteract the viral agent, but the virus mutates in the course of an infection, rendering the antibody ineffective. The AIDS virus, which resembles the visna virus in some genetic material, may profit from such variability, which is called antigenic drift. The virus is known to undergo steady genetic change, perhaps because of its very rapid and relatively inaccurate replication. Antigenic drift may account in part for the finding that the antibodies AIDS victims are able to produce have little neutralizing effect when they are tested in vitro against the virus.

The AIDS virus has little need for evasion, however; it avoids destruction by preemptively destroying the immune system. The phenomenon has parallels in other viral infections. Even measles temporarily weakens immunity, an effect noted in 1908 by Clemens von Pirquet. In people who have been exposed to tuberculosis and have acquired resistance to the disease the injection of a small amount of tubercle proteins under the skin produces an observable reaction. Von Pirquet noted that sensitive individuals frequently lost their skin-test reactivity during a bout of measles, although the reactivity returned later.

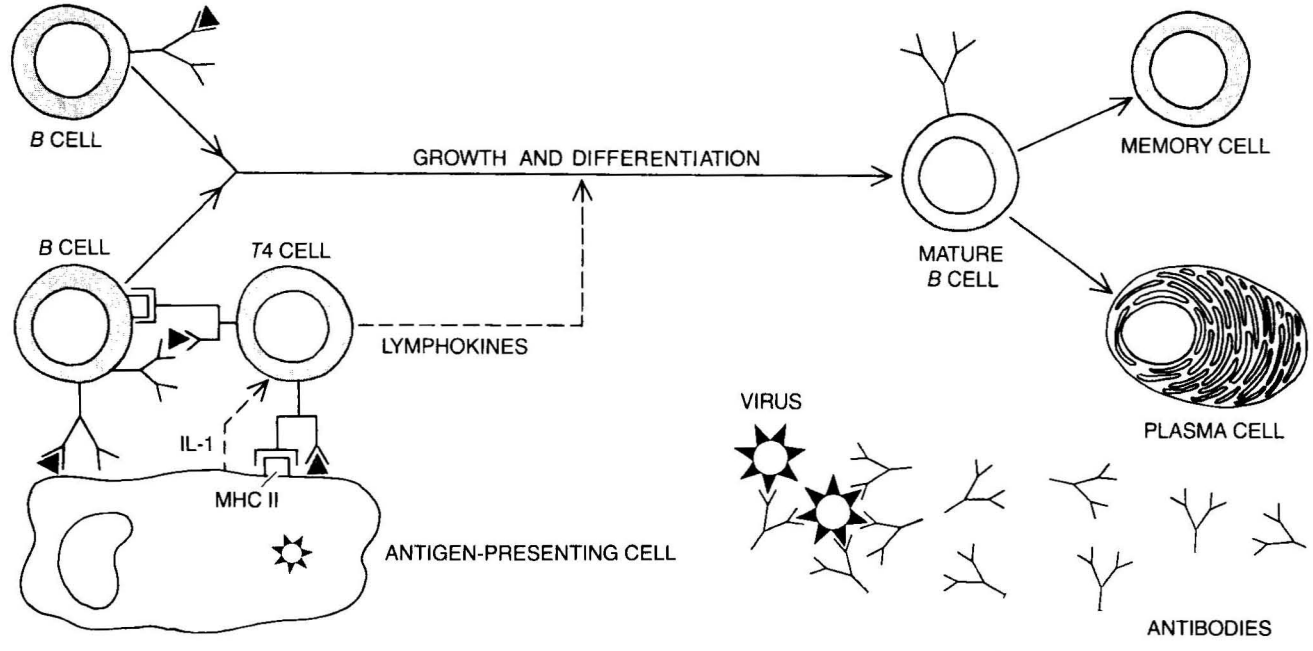
In contrast to this temporary loss of reactivity, certain viruses that infect animals produce a lasting depression of immune response. Many are retroviruses, a group that takes its name from a unique feature of its members' life cycle. In the usual biological sequence genetic information, carried on DNA, is transcribed into RNA, which is then translated into the proteins necessary for life. As retroviruses infect a cell they reverse the sequence: their genetic code, carried on RNA, is transcribed "backward" into DNA. In some cases the DNA is integrated into the host cell's chromosomes in the form of a sequence known as a provirus. Later the host cell transcribes the viral genes and synthesizes the proteins they encode, which are assembled into new viruses. Retroviruses can have a profound effect on the genetic makeup and therefore the properties of the cells they infect; many are known to cause cancers. Knowledge of the lasting immunosuppression produced by some animal retroviruses led to the suspicion, confirmed by the discovery of LAV and HTLV-III, that the cause of AIDS is a retrovirus.

*T*4-Cell Depletion

In the case of the AIDS retrovirus, immunosuppression results from viral infection of *T*4 lymphocytes, which in their role as inducer and helper cells orchestrate much of the immune re-



T-CELL COLLABORATION leads to the destruction of a virus-infected cell. An antigen-presenting cell such as a macrophage ingests virus, breaks it down and displays the antigenic viral proteins (*color*) on the cell membrane together with a Class II MHC molecule (*MHC II*), one of the macrophage's own proteins. A *T4* cell becomes activated when it binds simultaneously to the antigen and to the MHC protein; interleukin-1 (*IL-1*), a protein secreted by the macrophage, also plays a role in activating the *T4* cell. The *T4* cell then secretes interleukin-2 (*IL-2*). Interleukin-2 induces *T8* cells that have also recognized antigen (together with a Class I MHC protein) to proliferate. Some of the *T8* cells go on to kill infected cells displaying the viral antigen. Later other *T8* cells suppress this cytotoxic response (*colored arrow*), thereby turning off the immune defense when its job is done. To fulfill their cytotoxic and suppressor roles the *T8* cells require help from the *T4* cells, delivered either through soluble proteins or through direct contact with the *T8* cells.



T4 CELLS HELP *B* CELLS to secrete antibody against a viral antigen. A *T4* cell is activated by interleukin-1 (*IL-1*) after recognizing antigen together with a Class II MHC protein (*MHC II*) on a macrophage or other antigen-presenting cell. The *T4* cell then binds to a *B* cell that has also recognized antigen on an antigen-presenting cell. Contact with the *T4* cell stimulates the *B* cell to mature, multiply and differentiate into a clone of memory cells and a clone of plasma cells, which secrete antibody; antibody molecules bind to the virus, surrounding and inactivating it. Lymphokines secreted by the *T4* cell aid maturation. *B* cells can also recognize free antigen that circulates in the blood or the lymph (*as shown at top left*), but they still require *T*-cell help to mature, grow and differentiate.

sponse. The loss of such cells from the blood, lymph nodes, spleen and other tissues in which they are normally concentrated is one of the most striking and consistent findings in AIDS patients. Ordinarily *T*4 cells make up from 60 to 80 percent of the circulating *T*-cell population; in AIDS they can become too rare to be detected. Many viruses kill the cells they infect, usually by rupturing the cell membrane. In culture the AIDS virus instead appears to alter and ultimately slow the growth of infected *T*4 cells, while other kinds of *T* cells continue to multiply normally. In time the *T*4 cells are selectively depleted, although a small number may remain, harboring the virus in a latent state. Recent work by Robin Weiss of the Institute of Cancer Research in London shows that in surviving cells the AIDS virus can mask the *T*4 marker on the cell surface or prevent its display. As a result the *T*4-cell decline appears to be even more dramatic than it is.

The reduction of the *T*4-cell population has consequences that reflect the cell's central place in the immune system. Lacking *T*4-cell help, *B* cells are unable to produce adequate quantities of specific antibody to the AIDS virus or to any other infection. The cytotoxic *T*-cell response is similarly hampered. Suppressor *T* cells cannot fulfill their role either. The *B* cells of AIDS patients, for example, continuously secrete large amounts of nonspecific immunoglobulin (the class of proteins to which antibodies belong); they never receive the *T*-cell signal that ordinarily would shut them down.

With the loss of *T*4 cells the level of interleukin-2 falls, slowing the clonal expansion of mature *T* cells, which is normally induced by the lymphokine. The reduced production of interleukin-2 and gamma-interferon depresses the activity of natural killer cells and macrophages, which these proteins normally stimulate.

The propensity of the AIDS virus for infecting a single kind of cell sets it apart from other retroviruses, which tend to affect a range of cells. Recent work accounts for the specificity of the AIDS virus. Weiss and an independent group led by David Klatzmann at the Pasteur Institute showed that a region of the cell membrane associated with the *T*4 marker, the protein that distinguishes *T*4 cells from other lymphocytes, acts as a receptor for the virus. The region serves as an initial attachment point for the virus as it infects the lymphocyte.

The virus's preference for the *T*4 cell is not absolute, however; it is likely that macrophages, blood platelets and *B* cells serve as reservoirs of the virus. Infection of *B* cells, for example, may explain their continuous secretion of immunoglobulin. Other cells outside the blood may also serve as reservoirs: the endothelial cells lining the blood and lymphatic vessels, the cells of epithelium (skin and related tissues), the glial cells of the nervous system and nerve cells themselves. The ability of the AIDS virus to infect the central nervous system may account for the psychosis and brain atrophy that is common in patients. Cells outside the blood may lack the surface proteins that would enable the virus to invade them directly, but they may become infected when diseased *T*4 cells or macrophages fuse with them.

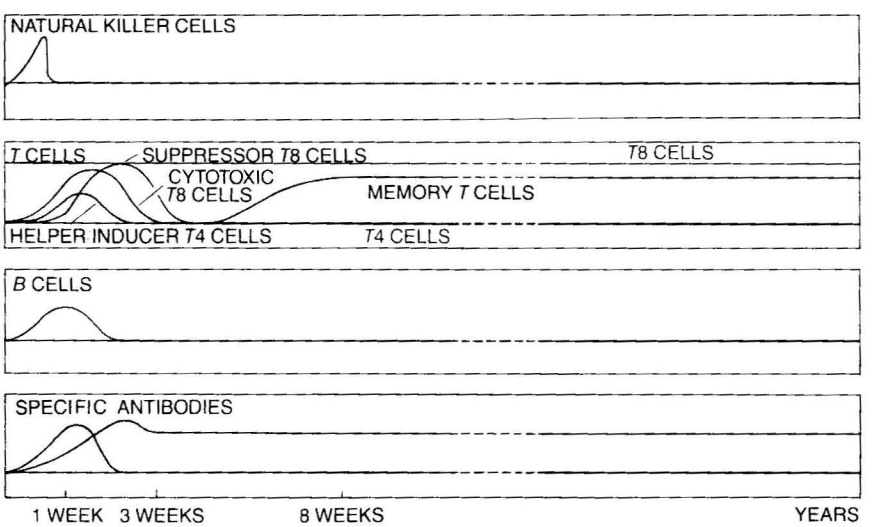
*T*4 cells may be most susceptible to infection when they have been stimulated and their numbers increased by chronic parasitic or viral infections. Infection with hepatitis *B*, Epstein-Barr virus or cytomegalovirus is common among several of the groups at risk for AIDS.

Soluble Suppressor Factor

A decrease in the number of *T*4 cells cannot account for the full extent of the immune defects seen in AIDS patients. In the early stages of the disease, for example, patients may still have a normal number of *T*4 cells, and yet their immune defenses are already severely weakened. Some workers have proposed that the virus elicits the production of anti-*T*4-cell antibodies, which besides killing *T*4 cells would also inhibit surviving *T*4 cells. Others suggest that cells previously infected by hepatitis *B* and carrying the viral genes in their DNA not only would be more susceptible to infection by the AIDS virus but also would respond differently to the infection.

The parallels between the AIDS virus and other immunosuppressive retroviruses suggested that a further change in *T*4-cell function occurs. Cats infected with feline leukemia virus (FeLV), a retrovirus, often die not from the leukemia but from other diseases, including some of the same opportunistic infections seen in AIDS. In 1978 Lawrence E. Mathes and Richard G. Olsen of the Ohio State University College of Veterinary Medicine found one basis of the immune suppression that leaves the cats open to such diseases.

Mathes and Olsen reported that a part of the FeLV protein envelope inhibits the response of cat *T* cells in culture to a substance that ordinarily causes the cells to proliferate, as they would in a normal immune response. The workers showed that the envelope molecule, which protrudes through the surface of an FeLV-infected cell, impairs the immune response of a living



IMMUNE SUPPRESSION is evident in a comparison of the responses to a measles infection typical of an AIDS patient (*color*) and of an individual with an intact immune system (*black*). The curves plot the activity of cells or antibodies over time. In a normal individual natural killer cells constitute the earliest response; their activity peaks within one or two days. The next response to intensify is that of *T* cells; their varieties (helper/inducer *T*4, cytotoxic *T*8 and suppressor *T*8), proliferate at different times, with the suppressor *T*8 cells being the last to peak. After suppression of the *T*-cell response a population of memory *T* cells persists, conferring immunity against a recurrence of the infection. Meanwhile *B* cells multiply and secrete antibodies. The various kinds of antibody reach their highest levels between one week and three weeks after infection. Suppression dampens the *B*-cell activity, leaving a small population of memory *B* cells, which maintain high levels of antibody for years. In AIDS patients *T*8 cells chronically outnumber *T*4 cells. Because of the reduced number and altered function of *T*4 cells, specific immune responses to infection do not ensue.

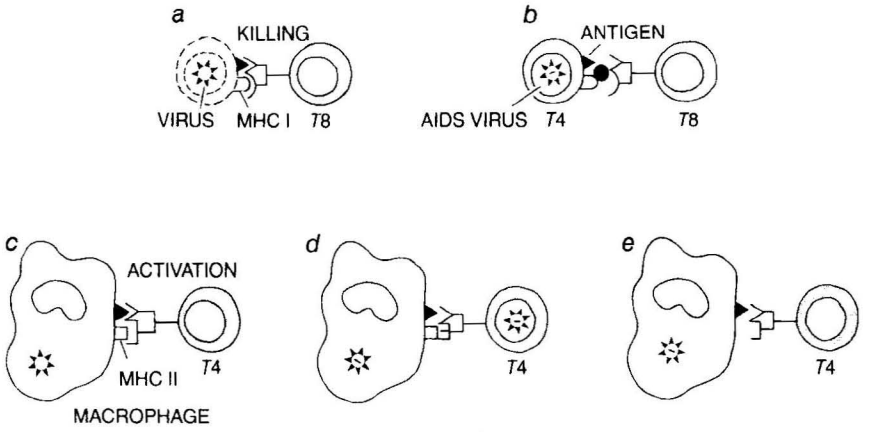
animal. They injected the purified protein, called p15E, into cats and found that the treated animals were more likely than normal cats to develop cancer after exposure to feline sarcoma virus, a tumor virus. Since then evidence has accumulated from other laboratories suggesting that many other mammalian retroviruses have protein components that produce immune deficiencies.

Such retroviral immune deficiencies share many of the features of AIDS, including a breakdown of all the responses that depend on *T*4 cells: antibody production, cytotoxic *T*-cell activity, *T*-cell proliferation induced by interleukin-2 and macrophage stimulation by gamma-interferon. Henry G. Kunkel and I, working at Rockefeller University, hypothesized that a protein factor might also play a part in AIDS. In 1982 we showed that blood cells cultured from patients suffering from AIDS release a factor that can inhibit certain immune responses. The substance, which we called soluble suppressor factor, blocks *T*-cell-dependent immune responses such as the production of specific antibody and *T*-cell proliferation both in vitro and when the factor is injected into mice.

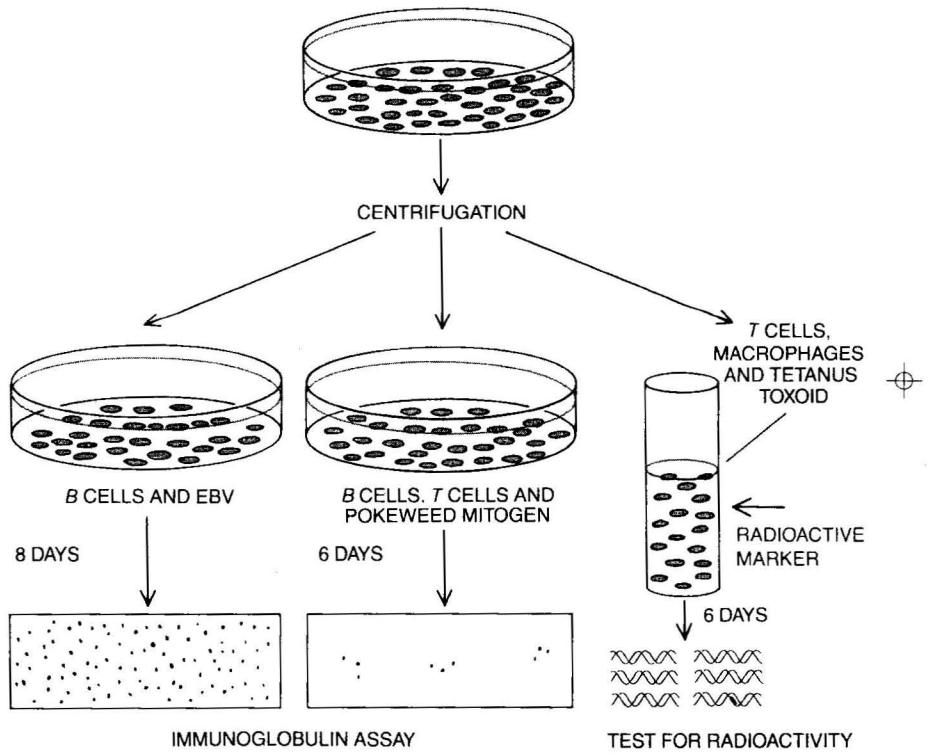
We went on to demonstrate that the source of soluble suppressor factor was infected *T*4 cells. We took *T*4 cells from a patient who was producing large quantities of the factor and fused them with normal *T* cells to immortalize them. The resulting clones of hybrid cells, we found, produced the suppressor factor in abundance. Later, working with Phillip D. Markham in Gallo's laboratory, we exposed normal *T*4 cells to the AIDS virus and showed that infection triggers secretion of a suppressor factor.

In an effort to determine whether our soluble suppressor factor is related to p15E, George J. Cianciolo and Ralph Snyderman of the Duke University Medical Center compared the sequences of amino acids making up the envelope proteins of the AIDS virus with those of p15E. They also included in the comparison another retrovirus with an affinity for human *T* cells: HTLV-I. A 26-amino-acid stretch of a protein in the HTLV-I envelope matched p15E in 73 percent of its amino acids. An envelope protein of the AIDS virus also bore a resemblance, looser but still close, to a region of p15E. One hypothesis, then, is that soluble suppressor factor originates in the envelope of the AIDS virus.

Malcolm A. Martin and his coworkers at the National Institute of Allergy and Infectious Diseases suggest another possible origin for soluble suppressor factor. They found that the



DISRUPTION OF ANTIGEN RECOGNITION may be one way the AIDS virus confounds the immune system. *T* cells must recognize antigen in conjunction with an MHC protein. Under normal circumstances a cytotoxic *T*8 cell destroys a virus-infected cell after simultaneously recognizing the viral antigen and Class I MHC molecule on the cell's surface (*a*). Hypothetically the AIDS virus could disrupt this process, protecting infected cells (mostly *T*4 cells) from recognition and destruction, by coding for an altered Class I MHC protein (*b*). The virus may also make an infected *T*4 cell itself unable to recognize antigen. *T*4 cells must bind both to antigen and to a Class II MHC molecule, displayed together on a macrophage (*c*), before they can induce *T*-cell proliferation and help the immune response. The virus may disrupt the *T*4 cell's receptor for MHC protein (*d*), causing recognition to fail. It could also incapacitate *T*4 cells by infecting macrophages and causing them to display reduced amounts of Class II MHC proteins, so that a *T*4 cell could not bind to antigen (*e*).



SOLUBLE SUPPRESSOR FACTOR produced by infected *T*4 lymphocytes from patients with AIDS or AIDS-related complex inhibits immune responses that depend on *T* cells. In the author's laboratory white blood cells from AIDS patients were grown in culture (*top*) and then spun in a centrifuge. The supernatant, or cell-free liquid, was added to three cultures of healthy cells. One contained *B* cells and Epstein-Barr virus (*EBV*), which ordinarily induces *B* cells to proliferate and secrete immunoglobulin without *T*-cell help. The response of the *B* cells to the EBV was not affected. The second culture contained *B* cells, *T* cells and pokeweed mitogen, a stimulus to which *B* cells can secrete antibody only with *T*-cell help. The *B* cells secreted little antibody. The third culture contained *T* cells, macrophages (needed to present antigen to the *T* cells) and tetanus toxoid. Under normal circumstances previously sensitized *T* cells proliferate in response to the tetanus antigen. Addition of a radioactively labeled nucleotide (a subunit of DNA) to the culture, followed by an assay of the amount of radioactivity incorporated into new DNA, indicated that the *T* cells had not multiplied. A factor in the supernatant evidently suppressed responses involving *T* cells.

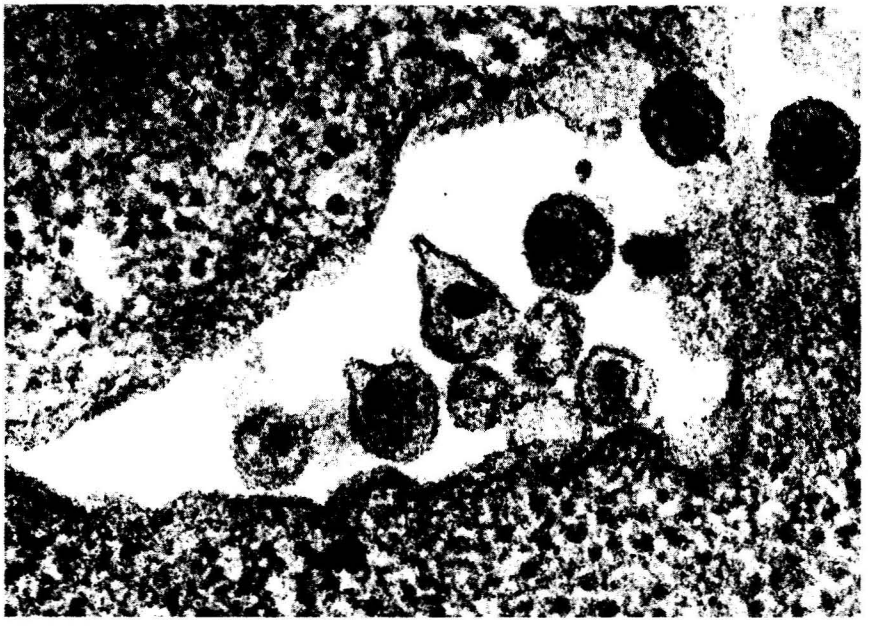
region of the FeLV genome coding for p15E and the region of the HTLV-I genome coding for the matching envelope protein share a resemblance to a segment of normal human DNA. The segment is thought to represent the genetic code of an endogenous retrovirus: a retrovirus that long ago became part of the human genetic makeup. It is possible that the AIDS virus causes the host cell to express the endogenous sequence (that is, to make the protein it encodes). Rather than encoding the soluble suppressor factor as part of its envelope gene, the AIDS virus would simply induce the synthesis of the factor by the host *T*4 cell. The same mechanism could also underlie the immunosuppression seen in other infections.

Either account of soluble suppressor factor is plausible, Cianciolo and Snyderman have shown. They synthesized a peptide (a short amino acid chain) that includes a series of amino acids common to p15E, the envelope sequences of HTLV-I and the AIDS virus, and the protein encoded by the human retroviral sequence. The 17-amino-acid peptide, the workers found, is capable of inhibiting the same responses as the intact, 196-amino-acid p15E molecule. The result strongly suggests that all the sequences have immunosuppressive effects.

Besides reducing the number of *T*4 cells and causing the release of a soluble suppressor factor from those that remain, the AIDS virus also makes the surviving *T*4 cells incapable of the crucial first step in the immune response: recognition of antigen. Anthony S. Fauci and H. Clifford Lane of the National Institute of Allergy and Infectious Diseases have shown, for example, that *T* cells from AIDS patients do not respond to a common bacterial antigen, tetanus toxoid. When the workers exposed *T* cells to the antigen in the presence of macrophages (needed to process and present the antigen), the cells did not proliferate, as normal *T* cells would.

A possible explanation is that the virus somehow impairs the receptor for antigen on the surface of *T*4 cells—the molecular lock into which a key consisting of antigen and a Class II MHC protein must fit to trigger the *T*-cell response. The virus might encode a protein that is expressed on the surface of the infected cell, intruding into and disrupting the receptor mechanism. Certain retroviruses that infect mice are known to disrupt receptors on *T* lymphocytes; as a result the *T* cells cannot single out antigen. In contrast to the incapacitated *T* cells in AIDS victims, however, the mouse lymphocytes kill infected and healthy cells indiscriminately.

A related mechanism could protect the AIDS-infected cells themselves from recognition by the immune system. A cancer-causing virus called Ad-12 blocks the transcription of certain genetic sequences in infected cells, causing the cells to express reduced amounts of Class I MHC proteins on their surface. Because cytotoxic *T*8 cells can only bind to antigen together with the host cell's own Class I MHC protein, the effect hinders the recognition and destruction of infected cells. There is some evidence that the AIDS virus acts similarly, thereby protecting the *T*4 cells and the other cells it infects. Such an effect would help the virus to elude any vestiges of a functioning immune system.



AIDS VIRUS PARTICLES, distinguished by their dark core of RNA, bud from the surface of infected *T* cells. The electron micrograph, made at the Pasteur Institute in Paris in 1983, is the first image of the earliest viral isolate, LAV; the following year the same virus was identified at the National Cancer Institute under another name, HTLV-III. The AIDS virus is a retrovirus: after entering a *T*4 cell it "reverse transcribes" its genetic code, carried on RNA, into the host cell's DNA. It subsequently induces the host cell to transcribe the retroviral genes and make RNA and proteins. The viral components form new viruses, which are then released from the cell. The micrograph has a magnification of 95,000 diameters.

Treatment

Where, in this tangle of pathology, might one intervene to cure the disease or lessen its effects? The question is an urgent one, not only because of the number of cases already diagnosed but also because AIDS as it is now defined probably includes only a very small proportion of the people actually infected with the virus. In recent years persistent, generalized enlargement of lymph nodes has become increasingly common among otherwise healthy members of the groups at highest risk for AIDS. Microscopic examination of the nodes following their surgical removal often reveals the depletion of *T*4 cells and the disruption of cellular architecture that is also found in full-blown cases of AIDS. It is thought many of these people will go on to develop first the AIDS-related complex (ARC), which is marked by unexplained fever, night sweats, and weight loss or chronic cough or diarrhea, and then AIDS itself.

Results of blood tests suggest an even larger number of people may carry the infection in spite of showing no sign of disease. The presence of antibody to the AIDS virus in the blood indicates prior exposure to the virus, and in many cases it also reveals infection: most homosexual men with antibody also have infectious virus in their blood, and in some cases also in their semen, saliva and tears. The prevalence of antibody among people at risk for the disease has led to estimates that between one and two million people in the U.S. alone are infected with the AIDS virus. Some of them may never show symptoms, even though retroviral infections persist for life. For others the incubation period may vary from several months to decades. By one estimate, which is probably conservative, 7 percent of the currently infected but still healthy individuals will develop AIDS each year.

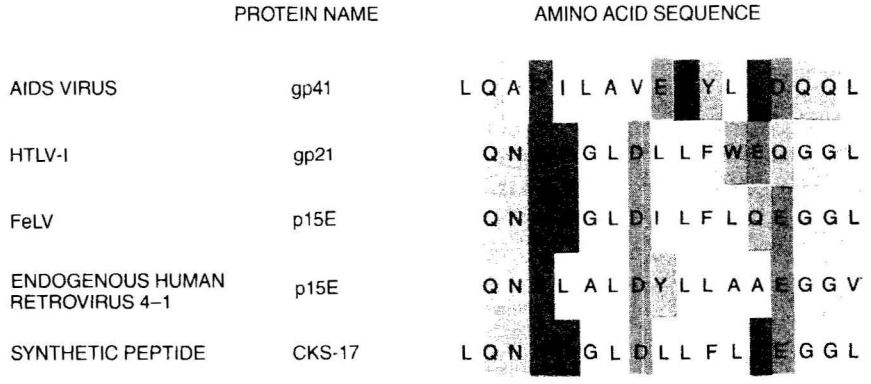
Equally troubling is the fact that these people represent a vast reservoir of carriers capable of spreading the

disease. Because a small fraction of AIDS carriers do not produce detectable amounts of antibody to the virus, current procedures for screening blood donors may not entirely eliminate the risk of infection from blood transfusions.

In treating active cases of AIDS physicians usually concentrate on the clinical manifestations of the disease: the infections and cancers that develop because of the immune deficiency. Long-term success is scant: almost every AIDS patient diagnosed as having an opportunistic infection dies within four years. Other treatment strategies have attempted to restore a measure of immune function. Bone-marrow transplants and injections of white cells have been tried in an effort to replenish the immune system with healthy cells. Patients have also been given interleukin-2 and interferons to stimulate their immune system. These efforts too have met with little success so far.

Another line of endeavor is a search for a weapon against the virus itself. Workers in Gallo's laboratory have found that the drug suramin, now used to treat protozoal infections, is capable in vitro of preventing the AIDS virus from infecting and damaging *T* cells. The needed concentration of the drug could be attained in the human body. Suramin acts by inhibiting the retroviral enzyme reverse transcriptase, which mediates the transcription of the viral RNA into host DNA (the first step in viral growth). Other drugs that counteract reverse transcriptase or interfere with later steps in the viral life cycle, the production of proteins from the viral genes and the assembly of new viruses, may also prove useful. Among the substances that have been proposed or tried are ribavirin, previously used against cold and flu viruses; the novel compounds HPA-23 and phosphonoformate; 3′-azido-3′-deoxythymidine, one of a family of anticancer agents; ansamycin, which is related to a drug given for tuberculosis, and alpha-interferon. Unfortunately many drugs that inhibit reverse transcription or viral replication also hinder the growth of the host's own cells including, ironically, those of the immune system.

In my laboratory at Cornell my colleagues and I are investigating antiviral drugs that have the added effect of stimulating the host's immune system. The compounds are related to guanosine, one of the building blocks of DNA and RNA; one basis for our interest in such substances is the fact that one of them, acyclovir, has proved successful against another viral infection, herpes simplex. In vitro the substances not only inhibit the replication of the AIDS virus but also increase the response of lymphocytes from AIDS patients to compounds that induce *T*-cell proliferation. The drugs, which seem to work by inhibiting enzymes that are crucial to the synthesis of viral RNA and DNA, are promising in themselves, and they emphasize the need to investigate interactions between antiviral drugs and the immune system.



AMINO ACID SEQUENCES of retroviral proteins that may suppress the immune system show close similarities. Each letter stands for one of the 20 amino acids that make up proteins; the shades of gray and color group amino acids with similar properties. Using a known immunosuppressive envelope protein from feline leukemia virus (FeLV), George J. Cianciolo and Ralph Snyderman of the Duke University Medical Center identified similar sequences in envelope proteins of the AIDS virus and HTLV-I (a virus that infects human *T* cells) and in a protein encoded by a segment of the human genome. The segment is thought to be an endogenous retrovirus. The investigators later synthesized a peptide containing amino acids shared by the sequences. The synthetic peptide suppressed immune responses as effectively as the FeLV protein, indicating that the other proteins are probably immunosuppressive also. Thus AIDS virus could produce a suppressor protein as part of its envelope or induce the cell to synthesize the protein encoded by the endogenous sequence.

An AIDS Vaccine?

For the growing ranks of the infected an effective antiviral drug is the best hope. The need for an AIDS vaccine is equally pressing. The genetic variability of the virus will hamper the search for a vaccine; samples of virus isolated from separate patients can differ by more than 30 percent in the RNA sequences encoding proteins that are thought to be key to recognition by *T* cells and antibody. A vaccine stimulates the immune system with an antigen, eliciting the production of antibody and the proliferation of memory cells. The variability of the AIDS virus means that subsequent exposure to the virus might not awaken the immunologic memory created by a vaccine. Nevertheless, it may be possible to identify invariant regions of the viral envelope to which antibody can bind effectively, and to use those regions as the basis of a vaccine. The recent development of the first effective vaccine against a mammalian retrovirus, FeLV, by Olsen and his associates has also encouraged investigators.

Research by William Haseltine and Joseph G. Sodroski at the Harvard School of Public Health suggests one approach to an AIDS vaccine. They showed that *T* cells infected with HTLV-I or the related virus HTLV-II secrete a regulatory factor that increases the transcription of the virus itself and of sequences in the host cell's DNA. The genome of the AIDS virus, they found, contains a sequence known as *tat*, which encodes a similar regulatory protein. In the case of the AIDS virus the protein might stimulate the transcription of viral genes (and perhaps the viral or host gene for soluble suppressor factor) while either inhibiting genes that stimulate replication of the *T*4 host cell or activating genes that turn off cell division. In addition to finding the region of RNA encoding the regulatory protein, Haseltine and Sodroski also identified the RNA sequence with which the protein interacts to stimulate transcription.

If the AIDS virus could be modified genetically by deletion of *tat* or the sequence with which the *tat* protein interacts, it could serve as a safe vaccine. It might elicit an immune response that would block a later infection with the unmodified virus, but the modified virus itself would not give rise to a widespread infection or deplete *T*4 cells. Alternatively, a drug that inhibited the synthesis of the regulatory protein encoded by *tat* could provide a chemical defense, as opposed to an immunologic one, against infection with AIDS. Either strategy for protection against the virus would be a boon to the tens of millions of people in this country alone who now are numbered among the

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The AIDS Virus

Part II of a two-part article on the human retroviruses. In 1984 the cause of AIDS was shown to be the third human retrovirus. Although knowledge of the virus was rapidly obtained, its toll will be heavy

by Robert C. Gallo

It is a modern plague: the first great pandemic of the second half of the 20th century. The flat, clinical-sounding name given to the disease by epidemiologists—acquired immune deficiency syndrome—has been shortened to the chilling acronym AIDS. First described in 1981, AIDS is probably the result of a new infection of human beings that began in central Africa, perhaps as recently as the 1950's. From there it probably spread to the Caribbean and then to the U.S. and Europe. By now as many as two million people in the U.S. may be infected. In the endemic areas of Africa and the Caribbean the situation is much worse. Indeed, in some areas it may be too late to prevent a disturbingly high number of people from dying.

In sharp contrast to the bleak epidemiological picture of AIDS, the accumulation of knowledge about its cause has been remarkably quick. Only three years after the disease was described its cause was conclusively shown to be the third human retrovirus: human *T*-lymphotropic virus III (HTLV-III), which is also called human immunodeficiency virus (HIV). Like other retroviruses, HTLV-III has RNA as its genetic material. When the virus enters its host cell, a viral enzyme called reverse transcriptase exploits the viral RNA as a template to assemble a corresponding molecule of DNA. The DNA travels to the cell nucleus and inserts itself among the host's chromosomes, where it provides the basis for viral replication.

In the case of HTLV-III the host cell is often a *T*4 lymphocyte, a white blood cell that has a central role in regulating the immune system. Once it is inside a *T*4 cell, the virus may remain latent until the lymphocyte is immunologically stimulated by a secondary infection. Then the virus bursts into action, reproducing itself so furiously that the new virus particles escaping from the cell riddle the cellular membrane with holes and the lymphocyte dies. The resulting depletion of *T*4 cells—the hallmark of AIDS—leaves the patient vulnerable to "opportunistic" infections by agents that would not harm a healthy person.

How HTLV-III manages to replicate in a single burst after lying low, sometimes for years, is one of the most fundamental questions confronting AIDS researchers. Another important question is the full spectrum of diseases with which the virus is associated. Although most of the attention given to the virus has gone to AIDS, HTLV-III is also associated with brain disease and several types of cancer. In spite of such lingering questions, more is known about the AIDS virus than is known about any other retrovirus. The rapidity of that scientific advance was made possible partly by the discovery in 1978 of the first human retrovirus, HTLV-I, which causes leukemia. In its turn the new knowledge is making possible the measures that are desperately needed to treat AIDS and prevent its spread.

DEGENERATING *T*4 CELL gives rise to a mass of newly made particles of the virus that causes AIDS: human *T*-lymphotropic virus III (HTLV-III), also called human immunodeficiency virus (HIV). The cell (magnified some 15,000 diameters) is the irregular treelike shape; the virus particles are the small, black specks. HTLV-III is a retrovirus: when it enters a cell, the RNA it carries as its genetic material forms the template for assembling a set of DNA genes. The DNA inserts itself among the cell's chromosomes, where it can remain latent until it is activated to make new virus particles. In the case of HTLV-III many particles are made at once, and the burst of replication may kill the host cell. Because the virus's chief host—the *T*4 lymphocyte—is a white blood cell that regulates the immune response, HTLV-III infection can cause profound immune deficiency.

The first sign that a new disease was afoot was the appearance of a rare cancer called Kaposi's sarcoma among the "wrong" patients. Kaposi's sarcoma is a tumor of blood-vessel tissue in the skin or internal organs that had been known mainly among older Italian and Jewish men and in Africa. In the late 1970's, however, a more aggressive form of the same cancer began to appear among young white middle-class males, a group in which it had been extremely rare. Many of the new Kaposi's sarcoma patients turned out to have a history of homosexuality, and these young men provided the basis for the first reports of a new syndrome, which came in 1981 from Michael S. Gottlieb of the University of California at Los Angeles School of Medicine, Frederick P. Siegal of the Mount Sinai Medical Center and Henry Masur of New York Hospital.

Seen mainly among young homosexual men, the new syndrome included opportunistic infections and a depletion of *T*4 cells as well as, in some cases, Kaposi's sarcoma. Soon epidemiologists at the U.S. Centers for Disease Control (CDC) noted a dramatic increase in pneumonia caused by *Pneumocystis carinii,* a widespread but generally harmless protozoan. It seemed clear that an infectious form of immune deficiency was on the rise, and the name AIDS was coined to describe it. AIDS was quickly found to be spreading among users of intravenous drugs, recipients of frequent blood transfusions and Haitians. A mysterious and fatal illness, apparently associated with life-style, had appeared.

Hypotheses about the cause of AIDS proliferated rapidly. It was suggested that the disease resulted from exposure to sperm or to amyl nitrate, a

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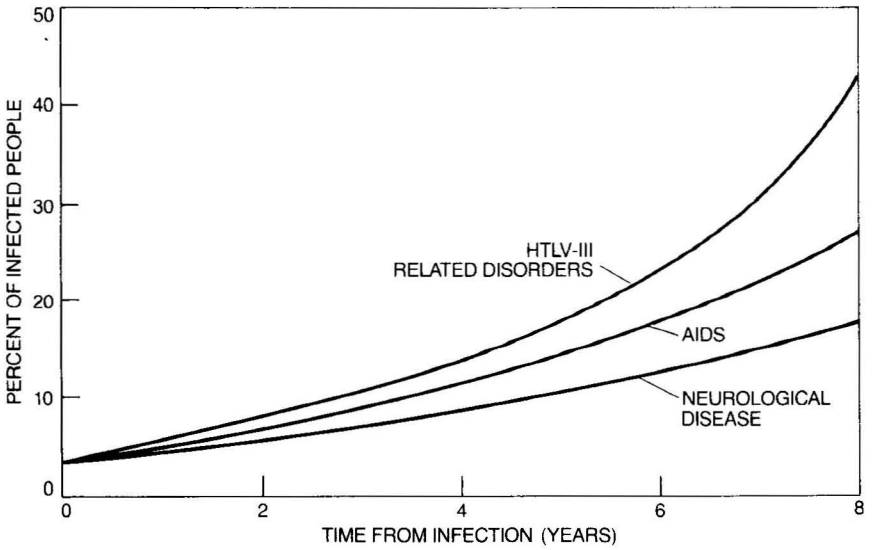
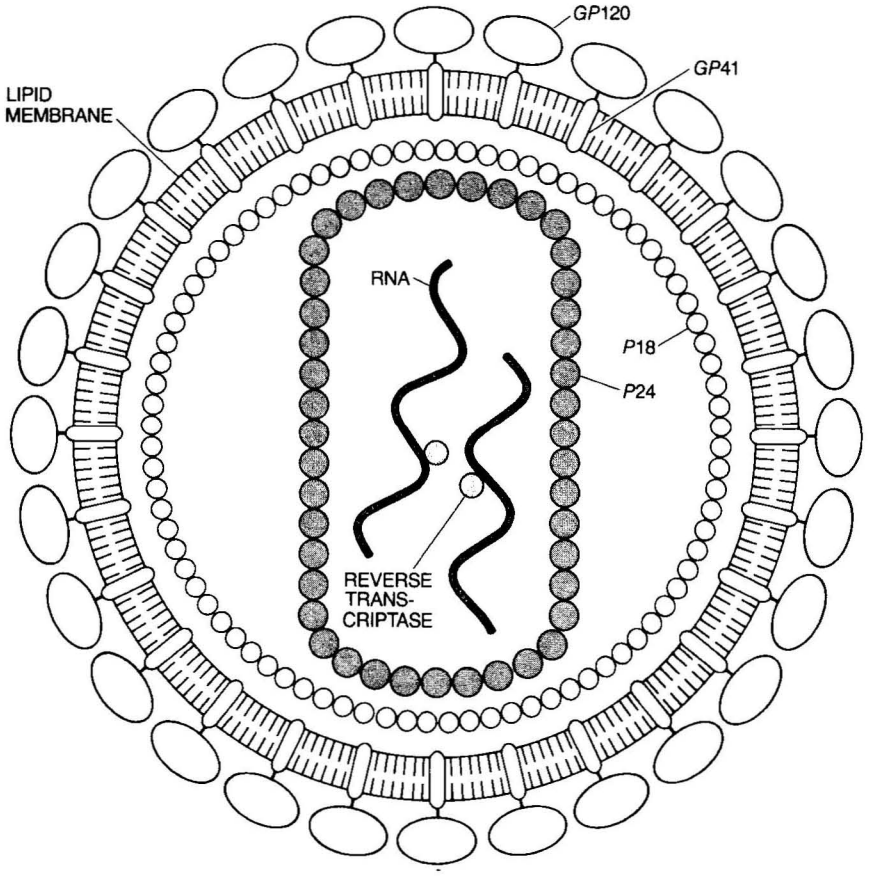


SPECTRUM OF DISEASES due to HTLV-III includes neurological disorders and cancer as well as immune deficiency. The curves show a hypothetical model for the proportions of infected people who might ultimately develop such illnesses. The neurological syndromes appear to be caused directly by HTLV-III infection of the brain, independent of secondary infections due to immune deficiency. The cancers are among a range of HTLV-III-related illnesses that may or may not be dependent on the immune deficiency.



HTLV-III VIRION, or virus particle, is a sphere that is roughly 1,000 angstrom units (one ten-thousandth of a millimeter) across. The particle is covered by a membrane, made up of two layers of lipid (fatty) material, that is derived from the outer membrane of the host cell. Studding the membrane are glycoproteins (proteins with sugar chains attached). Each glycoprotein has two components: *gp*41 spans the membrane and *gp*120 extends beyond it. The membrane-and-protein envelope covers a core made up of proteins designated *p*24 and *p*18. The viral RNA is carried in the core, along with several copies of the enzyme reverse transcriptase, which catalyzes the assembly of the viral DNA.

stimulant used by some homosexuals. It was even proposed that AIDS had no specific etiologic agent: the patients' immune systems had simply broken down under chronic overexposure to foreign proteins carried by other people's white blood cells or by infectious agents. Yet it seemed more plausible to think of a single cause, and several workers suggested known viruses such as Epstein-Barr virus or cytomegalovirus, which are members of the herpes virus family. Both were long-established viruses, however, whereas AIDS seemed to be a new disease. Moreover, neither virus has an affinity for *T* cells.

James W. Curran of the CDC and his colleagues, who had been following the nascent epidemic, clearly favored the notion of a new infectious agent. In late 1981, as I listened to Curran outline what was known about the epidemiology of AIDS, I was already in agreement with him. A clue as to what the new agent might be came from the fact that some hemophiliacs had developed AIDS after receiving infusions of a preparation called Factor VIII, prepared from the plasma of many blood donors. In preparing Factor VIII the plasma is passed through filters fine enough to remove fungi and bacteria—but not viruses.

That observation supported those who had argued in favor of a virus. Yet if one could not look to established viruses as the cause, how could the culprit be identified? Any virus that was a candidate would have to fit what was known about the agent, which included the following. It was present in whole blood, plasma and semen as well as in Factor VIII. The epidemiological pattern showed that it could be transmitted by sexual contact, blood and congenital infection. Infection led, directly or indirectly, to the loss of *T*4 cells.

As it happened, that pattern was familiar to me and my co-workers, because HTLV-I had been isolated in my laboratory in 1978. (Its story is told in the first part of this article: "The First Human Retrovirus," in last month's *Scientific American*.) HTLV-I can be transmitted by blood, intimate contact and congenital infection; it has a strong affinity for *T* cells. Furthermore, although the chief effect of HTLV-I is leukemia, the virus can also cause a mild immune deficiency in some patients. Accordingly, in the spring of 1982 I proposed that the cause of AIDS was likely to be a new human retrovirus.

To refine and test the retrovirus hypothesis I assembled a small working group of scientists, each chosen for a

cians, epidemiologists, immunologists and molecular biologists were investigators experienced in animal retrovirology. One of the retrovirologists, Myron Essex of the Harvard Medical School, had published results lending support to the idea that a human retrovirus might cause AIDS. Essex had shown that a retrovirus called feline leukemia virus (FeLV) can cause either leukemia or immune deficiency in cats. A minor variation in the virus's outer envelope, it was later shown, determines whether infection leads to immune suppression or to cancer.

These suggestive results made it seem even more plausible that a variant of HTLV-I (or its near relative HTLV-II, isolated in 1982) might be the AIDS agent. Essex's group and my own quickly began searching for such a virus. Soon we were joined by a third group, led by Luc Montagnier of the Pasteur Institute, who had been stimulated by the retrovirus hypothesis. All three groups employed the methods that my colleagues and I had developed for isolating HTLV-I: the virus was cultured in *T* cells stimulated by the growth factor called IL-2 and its presence was detected by sensitive assays for the viral reverse transcriptase.

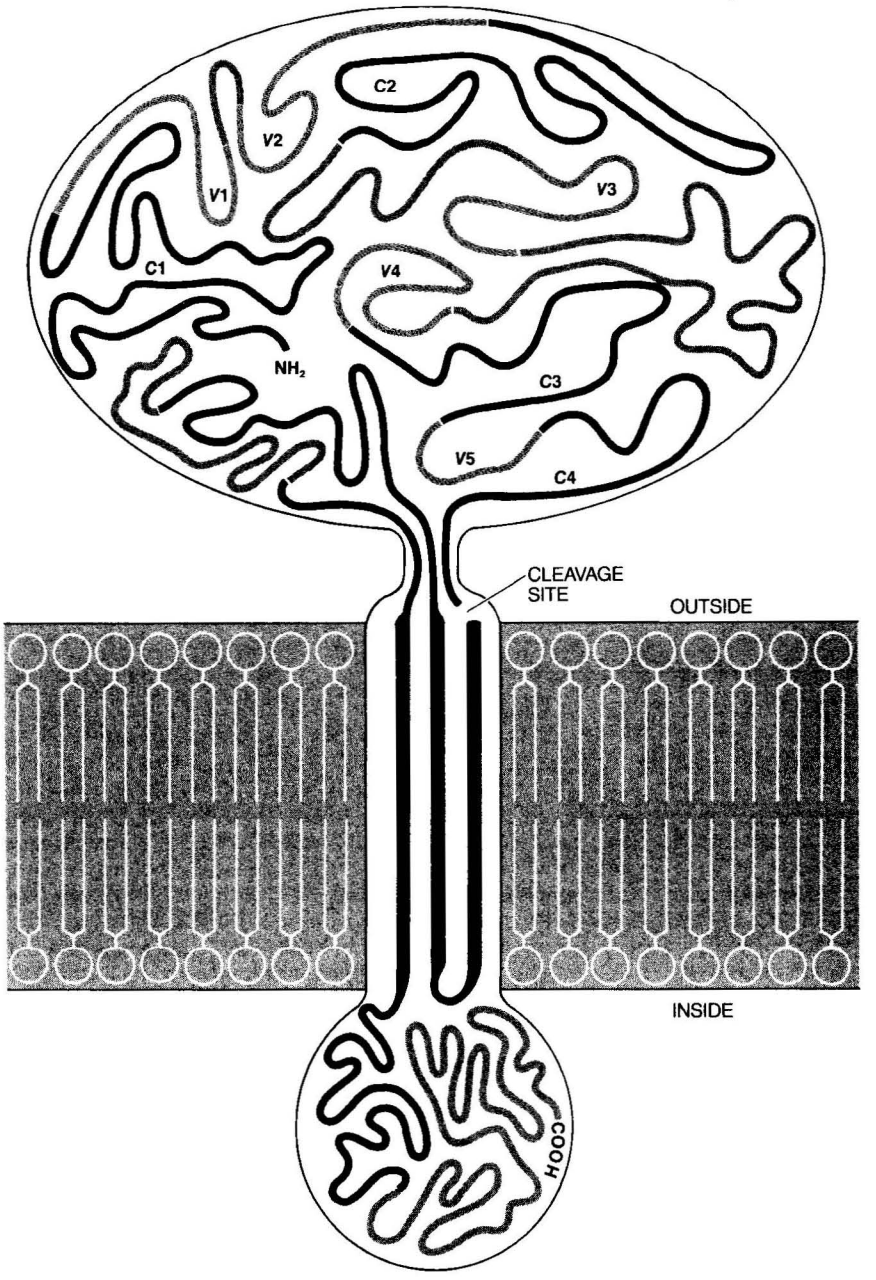
Those methods quickly produced results. Beginning in late 1982 and continuing throughout 1983 my coworkers and I found preliminary evidence of retroviruses different from HTLV-I or II in tissues from people with AIDS or pre-AIDS conditions. Then in May of 1983 Montagnier and his colleagues Françoise Barré-Sinoussi and Jean-Claude Chermann published the first report of a new retrovirus from a patient with the lymphadenopathy ("swollen glands") typical of some pre-AIDS cases. The French investigators later gave their find the name lymphadenopathy-associated virus (LAV).

The initial report of LAV was intriguing, but it was hardly a conclusive identification of the cause of AIDS. The reason is that the methods then available (reverse-transcriptase assays accompanied by electron microscopy) can show that a retrovirus is present in a tissue sample but cannot specify the precise type of virus. Unique identification is possible only if reagents (such as antibodies) are available that react with the proteins of that virus and no other. Making such reagents requires large quantities of purified viral proteins; to obtain them the virus must be grown in the laboratory.

The new virus (or viruses), however, resisted the early attempts at laboratory cells, the cells died. Hence no specific reagents to the new isolates could be made. We had previously learned how to culture HTLV-I and II, and reagents to those viruses were available. As a result it was possible to show that the viruses present in AIDS patients were not HTLV-I or HTLV-II, but through-

make a positive identification because specific reagents were lacking. Moreover, in the absence of reagents one could not say that any two of the new isolates were the same, which was clearly a requirement for showing that AIDS has a single cause.

The answer to such difficulties was



ENVELOPE GLYCOPROTEIN has an important role in HTLV-III's entry to its host and also in the death of the host cell. The protein includes some regions that are constant from one viral strain to another (*dark color*), some that are highly variable (*light color*) and others that are intermediate (*gray*). Entry to the cell seems to depend on an interaction between one or more of the constant regions and molecules in the cell membrane. The envelope protein is also involved in the budding of a new virus particle from the cell, which may leave a hole in the cell's surface. The model shown (one of several possible models of the protein's structure) was developed by Hans Wolf and his colleagues at the Max von Pettenkoffer Institute in Munich in collaboration with the author's group.

to find a way to grow the virus. In the fall of 1983 my colleague Mika Popovic identified several cell lines that could be infected with the virus but resisted being killed. To obtain them the blood cells of a person with leukemia were separated and allowed to proliferate into clones of genetically identical cells. Many clones were screened, and several were found to have the right combination of qualities; the most productive of them was the clone designated *H9*. All the resistant lines are made up of leukemic *T4* cells that are immortal in culture and therefore an endless source of virus.

Why certain *T4* cell lines should resist the cytopathic effects of the virus is a significant question that has not been answered. In the winter of 1983–84, however, my colleagues and I had little time for that puzzle because we were concentrating on growing the virus. By December substantial quantities were being grown, and soon afterward reagent production was under way. With reagents in hand, we could go back and identify the many stored viral isolates. Initial testing showed that 48 isolates from AIDS patients or members of risk groups were of the same type. In contrast, the virus so identified was not found in any members of a control group of 124 healthy heterosexuals.

Continuous production of the virus also yielded enough viral proteins to provide the basis of a blood test. (Although there are several methods of testing blood for the AIDS agent, all of them rely on the reaction between viral proteins and antibodies in the infected person's blood.) The first blood testing was done by my colleague M. G. Sarngadharan, working on serum identified only by a code. By means of such "blind" testing Sarngadharan found virus in the serum of from 88 to 100 percent of AIDS patients (depending on the study), in a high but varying proportion of people in risk groups and in almost no healthy individuals outside the risk groups. The cause of AIDS had been established.

My colleagues and I reported these results in a series of publications in May, 1984. The retrovirus we had identified showed an affinity for *T4* cells and also killed those cells. In accord with the prevailing conventions of virus nomenclature, the isolates were given the generic name HTLV-III and individual strains were distinguished by the initials of the patient from which they had come. Later it was shown that LAV is a different strain of the same virus. Later still, the name HIV was coined by a committee set up to resolve the problems caused by the existence of multiple names for the same biologic object.

Demonstrating the cause of AIDS was a fundamental step. Perhaps equally important from the viewpoint of public health was the fact that growing the virus had provided the basis for a practical blood test. The infected *H9* line was given to several biotechnology companies, which used it as a source of viral proteins for a commercial blood test. The commercial test, marketed in 1985, virtually eliminated the risk of contracting AIDS through blood tranfusion.

Although only three years have elapsed since the cause of AIDS was identified, much has been learned about how the virus gives rise to disease. When a person is first infected, his (or her) immune system does respond by making antibodies. That response is clearly not adequate, however, and the virus takes hold. In many cases lymphocytes then begin to proliferate abnormally in the lymph nodes. Thereafter the node's intricate structure collapses, and a decline in the number of lymphocytes in the node follows. Soon the number of lymphocytes in the blood also decreases, leaving the patient open to opportunistic infections [see "The Immune System in AIDS," by Jeffrey Laurence; *Scientific American*, December, 1985].

What events at the cellular level underlie this clinical catastrophe? It seems infection may be initiated by free virus or by virus carried in infected cells. Once the virus is inside the body its target consists of cells bearing the *T4* molecule in their outer membrane. That molecule defines the category of *T4* lymphocytes, but it is also found on cells called monocytes and macrophages, and it appears that *T4*-carrying monocytes and macrophages are among the first targets of infection by the AIDS virus.

Monocytes and macrophages arise from the same bone-marrow precursors as lymphocytes, but they have different roles in the immune response. Among the roles of the macrophage are interactions with *T4* lymphocytes that stimulate the *T4* cells to undertake their tasks. Some of the interactions occur in the lymph node, and observations by Peter Biberfeld of the Karolinska Institute in Stockholm and Claudio Baroni of the University of Rome suggest that many *T4* cells are infected in the lymph node during contact with a macrophage. After a variable latency the infected lymphocyte may be killed by viral replication.

Clearly the *T4* population is reduced by the death of infected cells. The effect is compounded by the fact that the killing halts the normal proliferation of the lymphocytes that accompanies their immune functions. In the interaction with a macrophage the *T4* cell not only is primed to respond to a particular protein but also is activated. Growth factors secreted by the macrophage cause it to begin a process of cell division that ultimately yields a clone of perhaps 1,000 descendants, all programmed to respond to the same antigen (protein). The descendants circulate in the blood and, on encountering the antigen they are programmed for, they induce the maturation of cells called *B* lymphocytes and *T8* cytotoxic cells that attack pathogens directly. In this way the "memory clone" provides part of the basis of lasting immunity.

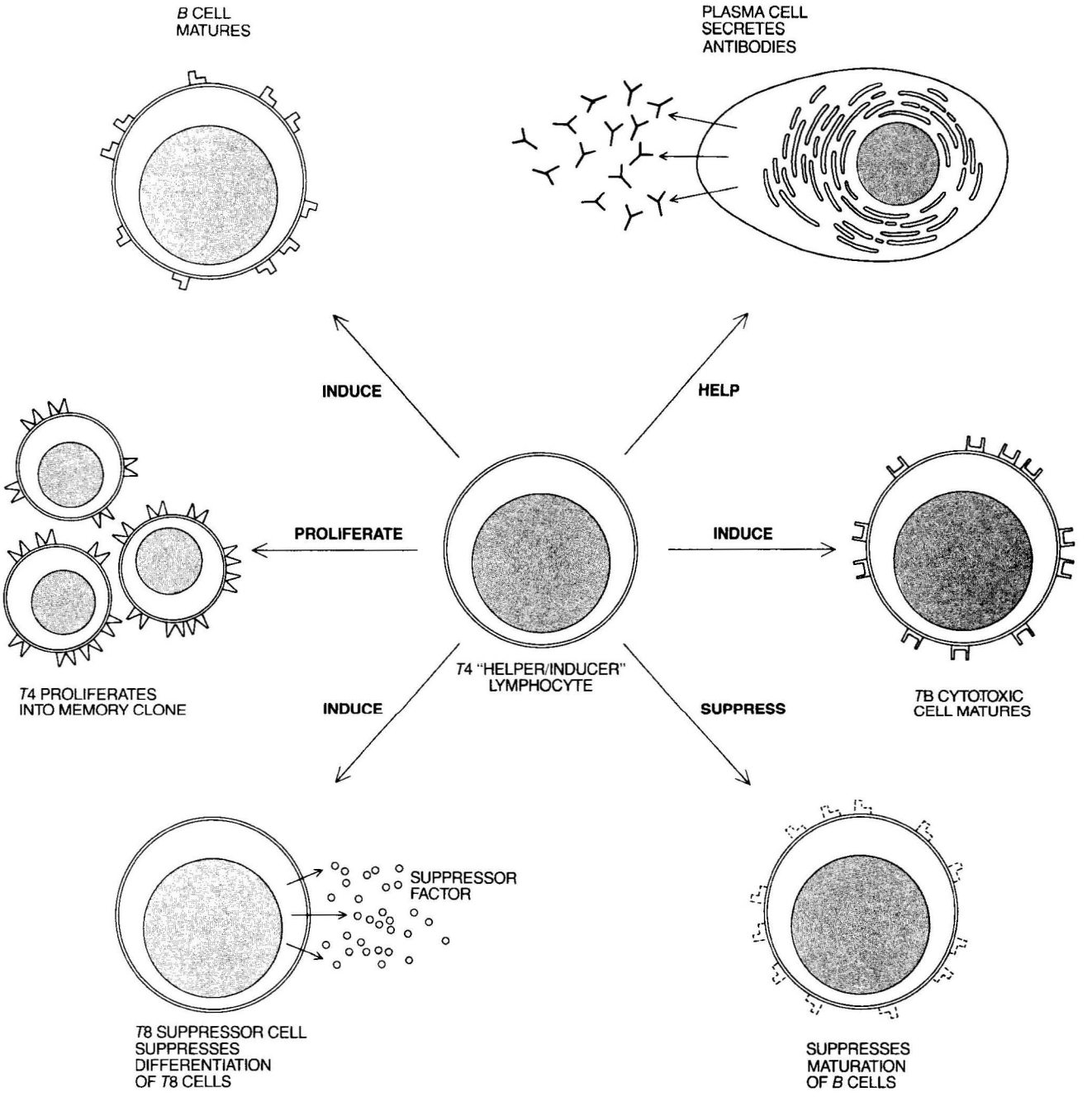
When a *T4* cell infected with the AIDS virus is activated, however, the result is quite different, as Daniel Zagury of the University of Paris has shown in collaboration with me. Instead of yielding 1,000 progeny, the infected *T* cell proliferates into a stunted clone with perhaps as few as 10 members. When those 10 reach the bloodstream and are stimulated by antigen, they begin producing virus and die. Other suggestions have been made, but I think the direct killing of infected lymphocytes and the abortive expansion of the memory clones are largely responsible for the profound depletion of *T4* cells observed in AIDS.

And what underlies these cellular events at the level of molecules? One of the most significant molecules in HTLV-III infection is *T4*. Indeed, by interacting with the outer envelope of the virus, *T4* may provide entry to the cell. The viral envelope consists of a membrane studded with glycoprotein molecules (proteins with attached sugar chains). Each glycoprotein has two subunits, called *gp41* and *gp120*. When HTLV-III makes contact with a cell, *gp120* appears to interact with a *T4* molecule in the cell's outer membrane. Thereafter the cell's membrane may form a vesicle that draws the virus into the cell. (This process, which is known as receptor-mediated endocytosis, provides entry to the cell for a variety of molecules needed for normal metabolism.)

The by now overwhelming evidence that *T4* is involved in infection was gathered in steps. The first step was the clinical observation that the infected cells are *T4* lymphocytes. Next, Robin A. Weiss of the Chester Beatty Laboratory in London, Angus Dalgleish of University College Hospital Medical

School and David Klatzmann of the Hôpital Salpêtrière found that antibodies to the *T4* molecule, which cover part of its structure, block HTLV-III infection. Then my colleagues and I found infected *T4*-carrying monocytes and macrophages. Some of the most telling evidence, however, came from Weiss and Richard Axel of the Columbia University College of Physicians and Surgeons, who inserted the *T4* gene into cells that do not ordinarily carry the marker and are not infected. Expression of the gene, entailing synthesis of the marker and its insertion into the cell membrane, is sufficient for infection of any human cell.

A similar, if less formidable, combination of evidence implicates *T4* in cell death. There too the initial observation was a clinical one: that the *T4*-lymphocyte population was depleted. In contrast to infection, however, the presence of *T4* alone is not sufficient for cell killing. Although monocytes and macrophages can be infected with HTLV-III, they are not easily killed, and the reason may be that they have few *T4* molecules on their surface. It seems that although a low level of *T4*



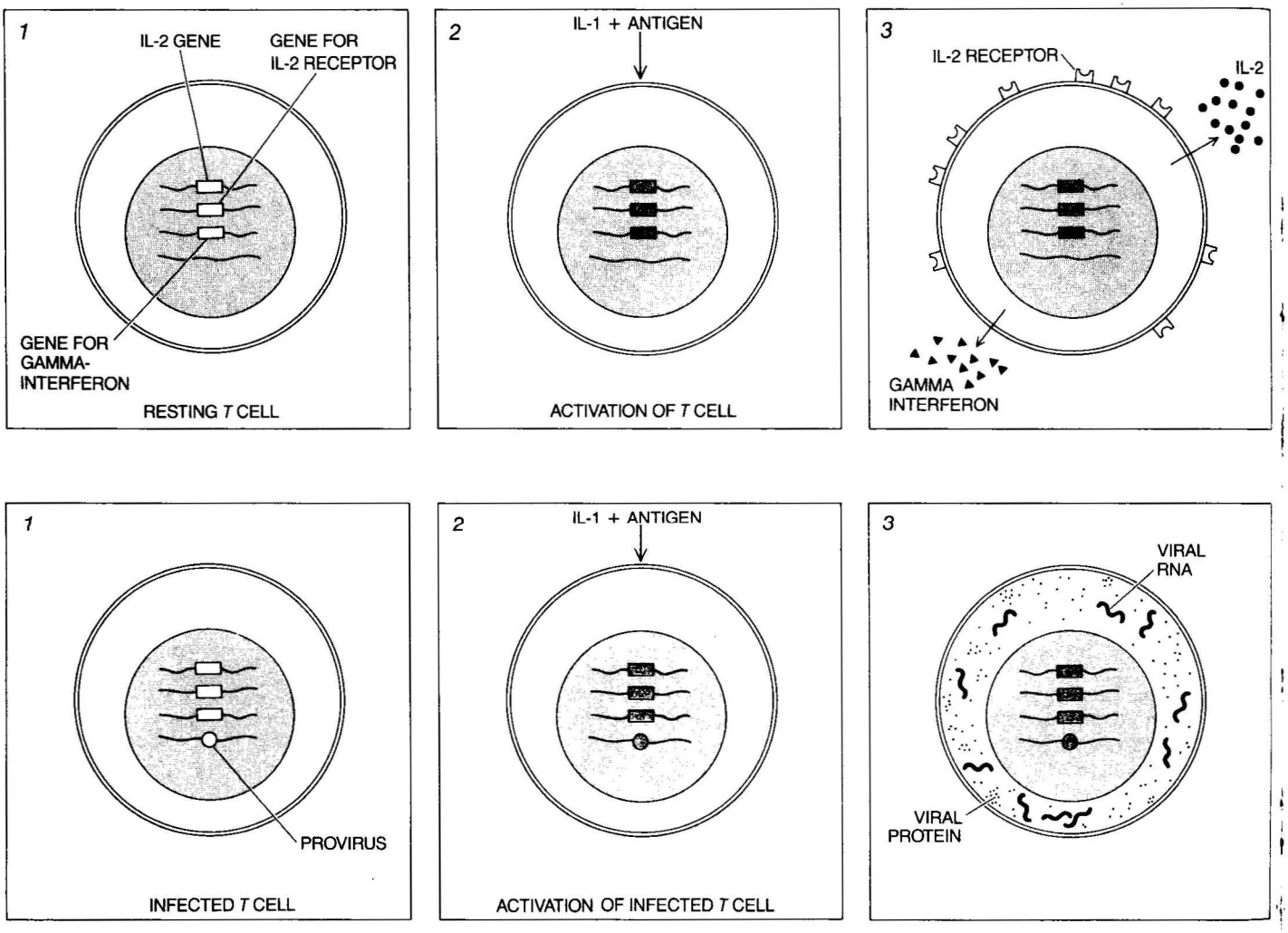
ROLES OF THE *T4* CELL in the immune response include interactions that prepare other cells to attack invading organisms. The *T4* cell produces substances that stimulate the maturation of the other main class of lymphocytes: the *B* cells. When a *B* cell matures, it differentiates into a plasma cell that secretes antibodies; *T4* cells may help them in that task. Other signals given off by the *T4* cell trigger the maturation of a second subset of *T* cells, the *T8* cells, that attack and kill cells infected by pathogens. When an infection has been brought under control, the *T4* cell has a role in suppressing further maturation of *B* and *T8* cells. As a final safeguard the *T4* cell proliferates into a clone of memory cells that circulate in the blood, ready to recognize a specific pathogen and carry out their multiple roles. As a result of these functions the *T4* cell is often referred to as the "helper/inducer" lymphocyte.

is sufficient for entry, a higher level may be required for the cytopathic effect. Indeed, as William Haseltine of the Dana Farber Cancer Institute has suggested, the rate of cell killing may be proportional to the concentration of *T*4 in the surface membrane of the infected cell.

Although no one knows why the death of the *T*4 cell should depend on the molecule that defines it, some suggestive findings make it possible to formulate a hypothesis. The killing depends not only on the *T*4 molecule but also on the viral envelope. My colleagues Flossie Wong-Staal and Amanda G. Fisher showed that mutant viruses lacking a piece of the inner end of *gp*41 have a drastically reduced cytopathic effect. Thus, like entry to the cell, its death may depend on an interaction between the viral envelope and the cell membrane. Perhaps that interaction (which takes place as the virus particle buds from the cell) punches a hole in the membrane. Because the virus buds in a mass of particles, the cell cannot repair the holes as fast as they are made; its contents leak out and it dies.

If that model (for the moment only a model) is correct, attention naturally falls on the question of how the virus is able to raise its rate of replication very rapidly from zero to a level high enough to kill the host cell. That question in turn focuses attention on the viral genome (the full complement of genetic information). The genome's DNA form, transcribed from the viral RNA and integrated into the cell's chromosomes, is called the provirus. The provirus includes the genes for the components of the virus particle, and in order for the virus to replicate, those genes must be expressed. How is gene expression controlled?

The answer (still very much under investigation) appears to lie in a group of regulatory genes whose presence renders the HTLV-III genome more complex than that of any other known retrovirus. The genetic complement of many retroviruses consists chiefly of the three genes that encode the components of the virus particle: *env* (which codes for the envelope proteins), *gag* (for the RNA-containing core) and *pol* (for the reverse transcriptase). Those three genes are flanked by stretches of DNA called the long terminal redundancies, or LTR's. The LTR's include DNA sequences that have a role in controlling the expression of the viral genes.



STUNTED MEMORY CLONE results from HTLV-III infection. The five upper panels depict the formation of a normal clone of memory *T*4 cells. Prior to infection the *T* cell is in a resting state (*1*). During infection a cell known as a macrophage secretes a protein called IL-1 and presents an antigen (a protein from the invading organism) to the *T*4 cell. The *T* cell is thereby immunologically activated. Several of its genes are turned on, including those for the growth factor IL-2 and its receptor (*2*). The activated cell secretes IL-2, and receptors for the protein appear on its surface (*3*). The binding of IL-2 to the receptors (*4*) initiates a process of proliferation that culminates in a memory clone with perhaps 1,000 members, each primed to react to the antigen with which

The genome of HTLV-III, however, includes at least four other genes, called *tat, trs, sor* and *3'orf*. Rather than encoding viral components, the four additional genes encode small proteins that help to regulate gene expression. The *tat* gene (discovered by Haseltine and his colleague Joseph Sodroski, and independently by Wong-Staal and Suresh Arya in my laboratory) has a dual function. Like its analogues in HTLV-I and II, *tat* appears to regulate the transcription of messenger RNA (mRNA) from the viral genes. In addition the *tat* protein affects events after transcription, perhaps the translation of the viral mRNA into proteins. The *trs* gene (discovered by Haseltine) appears to control the balance among the various forms of viral mRNA. The functions of *sor* and *3'orf* are unknown.

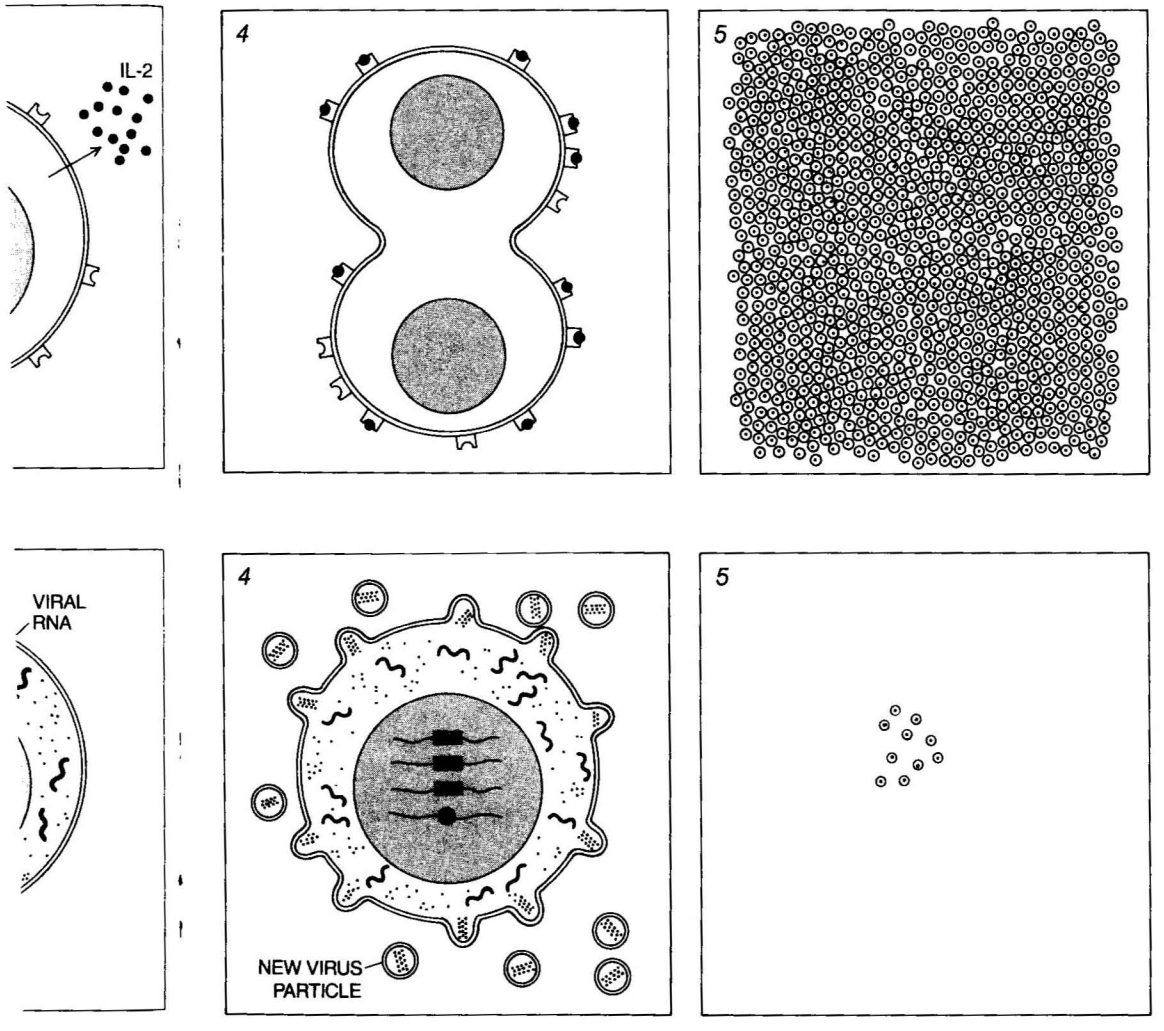
There are many other unknowns in this complex system, and it is too soon to say confidently how it works. It is not too soon, however, to hazard a general hypothesis, taking as a premise the fact that the virus does not replicate until the *T* cell is immunologically activated. The LTR's of the AIDS virus share some DNA sequences with the cellular genes that are turned on during immune activation. I think the chemical signals that activate the *T*4 cell simultaneously activate the viral LTR's. Somehow the small regulatory proteins interact with the provirus to boost synthesis of the viral components very quickly. The components self-assemble and bud from the cell in a pulse that may kill the host.

In summary form that is what is known about how HTLV-III cripples the immune system. Although most of the attention given to the virus has been devoted to that process, it has become increasingly clear that immune deficiency is only one effect of the AIDS agent. The other main type of disease caused by HTLV-III is seen in the central nervous system. HTLV-III was first detected in brain and spinal-cord tissues from AIDS patients by my colleagues George M. Shaw, Beatrice Hahn, Wong-Staal and me in 1984. The infected cells appear to have some of the properties of monocytes and macrophages. Those cells may be able to cross the blood-brain barrier, which separates the central nervous system from the blood supply; perhaps macrophages become infected in the blood and transport the virus from there to the brain.

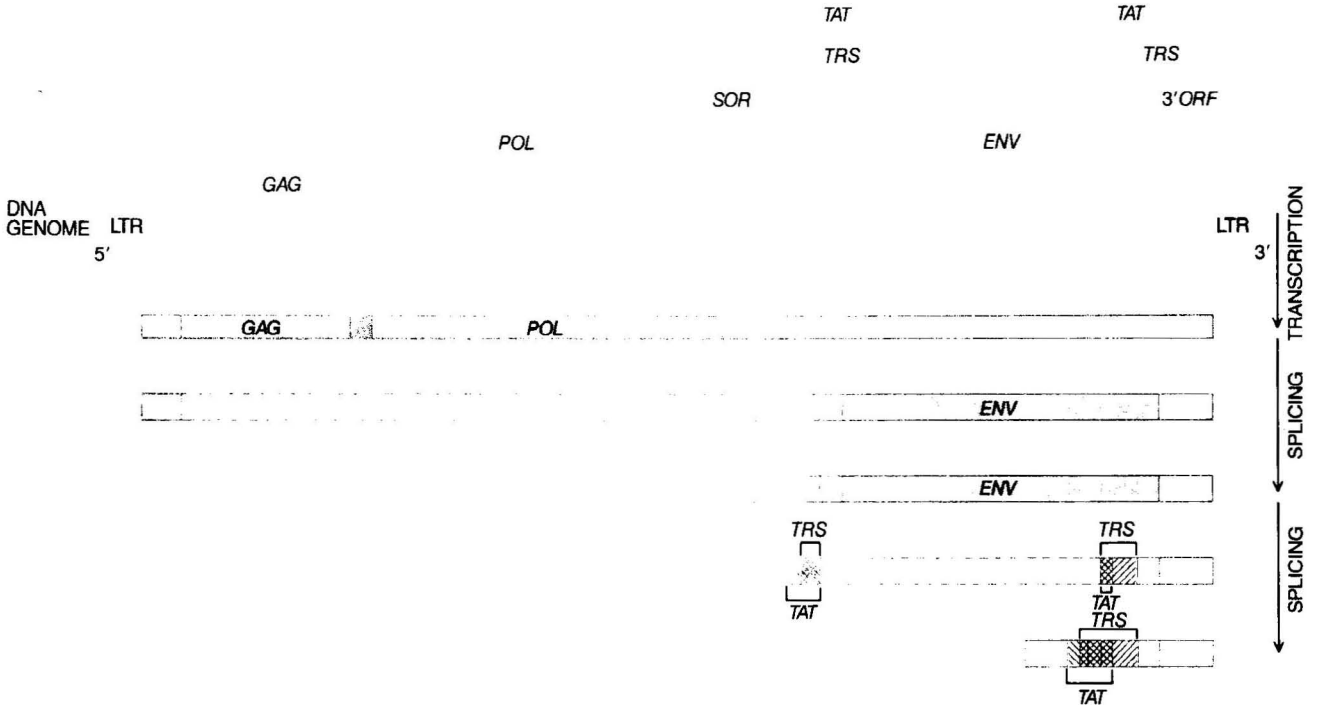
In the brain and spinal cord the virus appears to have a direct pathogenic effect that is not dependent on the immune deficiency. The chief pathologies observed in the brain are an abnormal proliferation of the glial cells that surround the neurons and lesions resulting from loss of white matter (which is, along with gray matter, one of the two main types of brain tissue). How the virus causes such anatomical changes is not understood. Nor is it known how the relatively limited range of structural aberrations due to the virus gives rise to a wide range of symptoms including dementia and mimicry of other neurological syndromes such as multiple sclerosis.

Whereas the neurological effects of HTLV-III are distinct from immune deficiency, the third main type of pathology—cancer—has a more ambiguous relation to the crippling of the immune system. People infected with the virus have an increased risk of at least three types of human tumor: Kaposi's sarcoma, carcinomas (including skin cancers often seen in the mouth or rectum of infected homosexuals) and *B*-cell lymphomas, which are tumors originating in *B* lymphocytes.

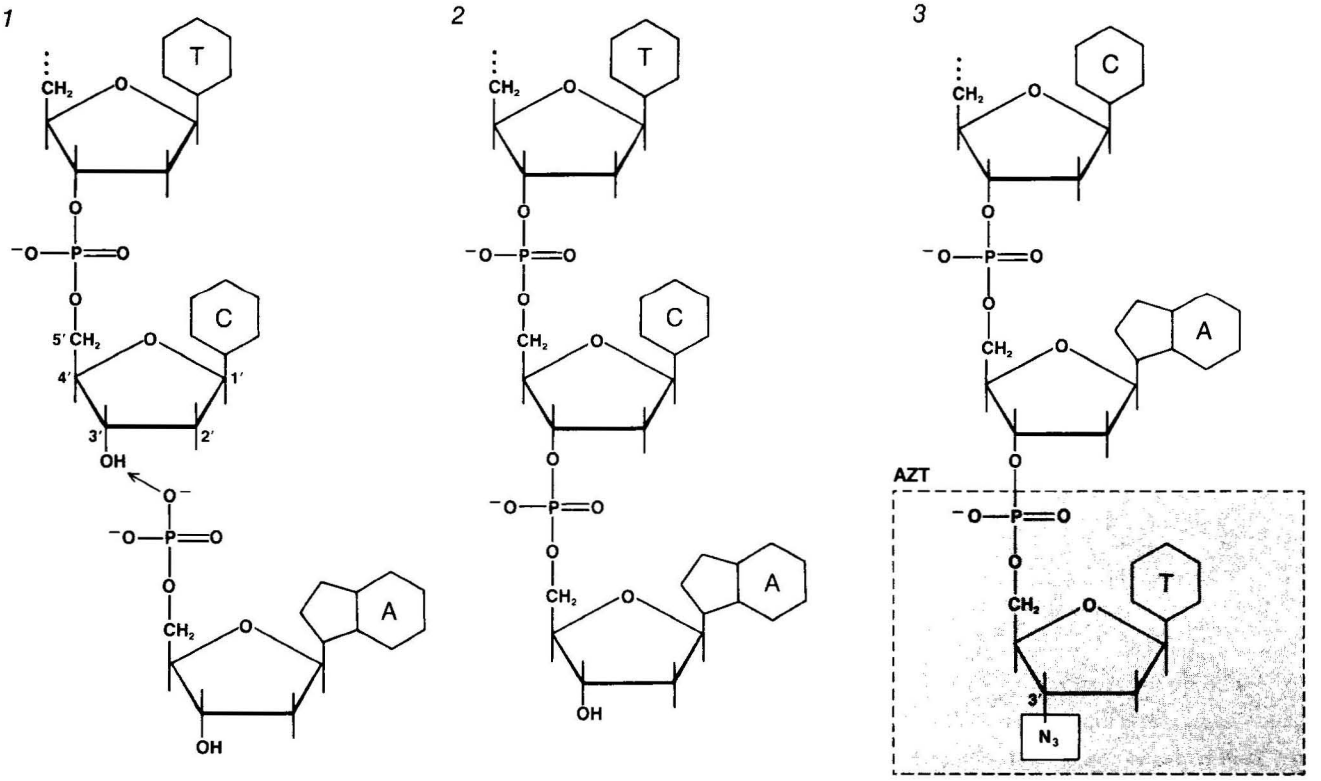
In some instances the tumors appear to be independent of immune deficits, as is suggested by the fact that homosexuals may have an increased risk of developing Kaposi's sarcoma even if they are not infected with the AIDS virus. Pathogens other than HTLV-III—perhaps sexually transmitted agents—are likely to be involved in these tumors. Yet infection with HTLV-III greatly increases the risk that Kaposi's sarcoma will develop. Therefore it seems likely that depression of the immune response enables secondary tumor-causing agents to infect and replicate freely. What they are is not known, but one may be human *B*-lymphotropic virus (HBLV), a new DNA-



the process began (*5*). The lower panels show what happens when an infected cell is activated. The viral DNA in the cell's chromosome is called the provirus (*1*). In the interaction with the macrophage (*2*) the provirus is activated along with the cellular genes. Viral RNA and proteins are made (*3*). They self-assemble into particles that leave the cell, often killing it (*4*). Hence the memory clone may have only 10 cells (*5*). This model may explain the loss of *T*-cell-based immunity in people infected with HTLV-III.



GENOME OF HTLV-III (its full complement of genetic information) is more complex than that of any other known retrovirus. At each end of the provirus (*blue*) are DNA sequences called long terminal redundancies (LTR's), which have a regulatory role. Between the LTR's are at least seven genes. Three code for viral components: *gag* for the core proteins, *pol* for reverse transcriptase and *env* for the envelope proteins. Four unusual genes—*tat*, *trs*, *sor* and 3'*orf*—encode proteins that help to control the expression of viral genes. The RNA made from the provirus (*red*) is spliced to yield the array of messenger RNA's (mRNA's) from which viral proteins are assembled. The core proteins and reverse transcriptase are made from an mRNA corresponding to the entire genome. One splice yields the envelope-protein mRNA, a second the small mRNA from which the *tat* and *trs* proteins are made.



DNA CHAIN IS TERMINATED by azidothymidine (AZT), the first drug to treat the symptoms of AIDS effectively. DNA consists of subunits called nucleotides, each of which includes a five-carbon sugar molecule. Normally a hydroxl group (OH) is present on the carbon designated 3' (*1*). The hydroxl group provides the attachment point for the next nucleotide by taking part in the formation of a linkage called a phosphodiester bond (*2*). AZT is an analogue of the usual nucleotides. The viral reverse transcriptase will incorporate it into a DNA chain (*3*). Because AZT lacks the 3' hydroxyl group, the chain is terminated, yielding an inactive provirus.

family recently isolated by my colleagues Zaki Salahuddin, Dharam Ablashi and Biberfeld and me.

The welter of pathologies caused by HTLV-III seems daunting, but the knowledge already gained about the virus has begun to lay the groundwork for treatment and prevention. The most promising therapies currently under investigation are based on interrupting the reverse transcriptase as it assembles the viral DNA destined to become the provirus. The drugs used for this purpose are chemical analogues of the nucleotides that form the subunits of DNA. When the analogue is supplied to an infected cell, reverse transcriptase will incorporate it into a growing DNA chain. Because the analogue lacks the correct attachment point for the next subunit, however, the chain is terminated. The truncated DNA cannot integrate itself into the chromosomes or provide the basis for viral replication, and so the spread of infection is halted.

Recent tests of azidothymidine, or AZT, have shown that this strategy can reduce mortality among AIDS and pre-AIDS patients as well as moderating their symptoms. AZT was formulated some 20 years ago as an anticancer drug. Although a failure in that role, it was resurrected in 1984 as a possible means of treating AIDS. After initial studies of the interaction of AZT and the viral reverse transcriptase, the drug was brought to the threshold of clinical use by Samuel Broder and Robert Yarchoan of the National Cancer Institute. Recently a multicenter trial was interrupted to begin wide distribution of AZT as a result of the dramatic benefits observed in the tests. It is not known, however, how toxic AZT may prove to be when it is used for a long period.

Perhaps the most important work now being done in the effort to curb AIDS is the development of a vaccine. For a vaccine to be effective it must safely evoke two different types of immunologic response. The *B* cells must be stimulated to produce neutralizing antibodies, which bind to the virus's envelope and prevent it from entering cells. In addition the cellular system anchored by the *T* cells must be capable of attacking and destroying cells already infected with the virus. Although, as I have mentioned, people infected with HTLV-III do make antibodies to the virus, the amount of effective, neutralizing antibody is worrisomely low, and of course cellular immunity is subverted by the death of the *T*4 cells. A successful vaccine must boost both reactions greatly.

AFRICAN GREEN MONKEY (*Cercopithecus aethiops*) may have harbored the ancestor of the AIDS virus: simian *T*-lymphotropic virus III (STLV-III). STLV-III does not usually cause disease in its simian host, but it might have infected humans and given rise to HTLV-III after many genetic changes. The monkey virus was isolated in 1985 by Myron Essex and Phyllis J. Kanki of the Harvard Medical School. The photograph is by Kanki.

That task is made considerably more complex by the virus's great genetic variability. Unlike many viruses, which have only a few strains, HTLV-III comprises a great many variants that form a continuum of related strains. Some pairs of variants differ by as few as 80 nucleotides of the 9,500 making up the viral genome; others differ by more than 1,000 nucleotides. Since the nucleotide sequence of the genome constitutes the genetic code for the viral proteins, such differences translate into variations in protein composition. Differences in proteins may in turn account for variations in biological activity seen among strains of HTLV-III, including preferences for infecting either *T*4 cells or macrophages.

Intriguingly, Wade P. Parks of the University of Miami (collaborating with Shaw and Hahn in my group) showed that an individual infected with HTLV-III may harbor several strains of the virus, all closely related in their genetic makeup. The fact that all the coexisting strains are closely related suggests that somehow their presence may "vaccinate" the infected person against reinfection by more distantly related strains. This pattern offers hope that a synthetic vaccine may be able to do the same. As yet, however, no manmade vaccine has been able to cope with the profusion of strains. My group and others are working on many approaches to a vaccine, some of which have yielded neutralizing antibodies. Yet so far the vaccines have been type-specific, neutralizing many but not all HTLV-III variants.

The progress made in only three years—identification of the cause of AIDS, formulation of a blood test, the first effective therapy and the beginning of vaccine development—is striking, particularly in view of the fact that AIDS is a viral illness, a type that has generally resisted effective therapy. Yet even if therapy and vaccine are brought into being on the fastest possible schedule, HTLV-III's toll will be heavy: many of the millions already infected will become ill before treatment is available.

The proportion of infected people who do go on to become ill may be considerably higher than was once thought. Along with Mark H. Kaplan of North Shore University Hospital on Long Island, Robert R. Redfield of the Walter Reed Army Institute of Research has led the way in developing

clinical categories that go beyond classical AIDS to consider HTLV-III infection in its full context. Redfield has developed a six-stage system of classification beginning with a positive blood test and ending with full-blown AIDS. Recently he used that system to follow a group of patients for as long as 36 months and found that about 90 percent of them progressed from the stage in which they began the study to a subsequent stage. Such results suggest that, contrary to what has been suggested, there may not be a large group of infected people who remain without symptoms.

It is difficult to say what the final toll will be. Regardless of its size, however, much of it will be felt in Africa. In some African countries epidemiological results show that a sizable fraction of people in the sexually active age groups are already infected. The high prevalence of infection in Africa is due partly to the fact that universal testing of the blood supply is beyond the economic reach of most African countries. As a result the virus is still being transmitted by contaminated blood. In addition, it appears that the virus has had more time to spread in Africa than it has had in any other part of the world.

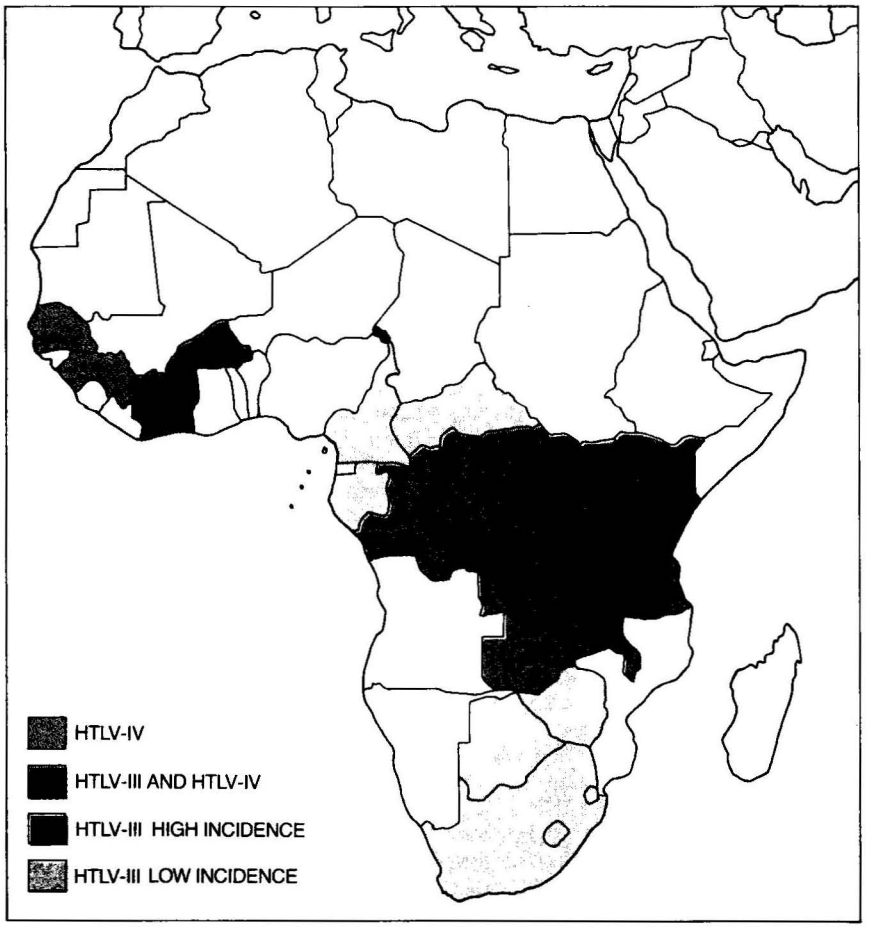
Recent results have begun to provide a picture of how the AIDS virus may have come to be. In 1985 Essex and his colleague Phyllis J. Kanki isolated a virus related to HTLV-III in African green monkeys, whose range includes much of equatorial Africa. The monkey virus, which is called simian *T*-lymphotropic virus III (STLV-III), may well be an ancestor of the AIDS agent. Yet although STLV-III is a closer relative of HTLV-III than any other animal retrovirus is, the relation between them is still not particularly close. Nor is the monkey virus pathogenic in its usual host.

Recently, however, the gap between the simian virus and the human one has begun to be filled by the discovery of a group of intermediate viruses. The first of these, called HTLV-IV, is closely related to STLV-III and is nonpathogenic, but it infects humans. It was isolated by Essex and Kanki in West Africa in 1985. More recently two viruses that are closely related to HTLV-IV but do cause immune deficiency have been discovered in the same region. Called LAV-2 and the SBL virus, they were isolated by the Pasteur group and by a Swedish group respectively. A plausible hypothesis is that STLV-III somehow entered human beings, initiating a series of mutations that yielded the intermediate viruses before terminating in the fierce pathology of HTLV-III.

That those fierce effects are of recent origin is shown by tests done on stored blood serum from many parts of the world. Tests on sera from the 1960's and 1970's detect no antibodies to HTLV-III anywhere except in a small region of central Africa, where the earliest signs of infection have been found in serum samples taken in the 1950's. It appears that after remaining localized for some time, the virus began spreading to the rest of central Africa during the early 1970's. Later in that decade it reached Haiti and may have reached Europe and the Americas from there.

Analysis of the origin and spread of HTLV-III leads to a conclusion that cannot be sufficiently emphasized: AIDS is not a disease of homosexuals or drug addicts or indeed of any particular risk group. The virus is spread by intimate contact, and the form of contact seems to be less important than the contact itself. Rapid spread of the virus depends on the accumulation of a pool of infected people that is large enough for a few exposures to result in infection. The pool need not consist of homosexuals or drug addicts. In Africa the pool is made up of heterosexuals, and Redfield, Kaplan and others have demonstrated heterosexual transmission in the U.S. Until a reliable vaccine is developed, intelligent caution and an understanding of the virus are the best weapons against its spread.

Does this terrible tale have a moral? Yes. In the past two decades one of the fondest boasts of medical science has been the conquest of infectious disease, at least in the wealthy countries of the industrialized world. The advent of retroviruses with the capacity to cause extraordinarily complex and devastating disease has exposed that claim for what it was: hubris. Nature is never truly conquered. The human retroviruses and their intricate interrelation with the human cell are but one example of that fact. Indeed, perhaps conquest is the wrong metaphor to describe our relation to nature, which not only surrounds but in the deepest sense also constitutes our being.



EPIDEMIOLOGICAL RESULTS indicate that HTLV-III and its relatives spread first in Africa. The map shows results of blood testing done in 1985 and early 1986. HTLV-IV, a nonpathogenic human virus closely related to STLV-III, is one of a group of viruses that may be intermediate between STLV-III and HTLV-III. The AIDS virus, still most prevalent in central Africa, has spread to the rest of Africa, Europe and both Americas.

1/22/88 Wall St. Journal

U.S., Japanese Negotiators Deadlocked On Tapping Each Other's Technology

By Eduardo Lachica
Staff Reporter of The Wall Street Journal

WASHINGTON — American efforts to get Japan to be more generous in sharing the fruits of Japanese research laboratories have reached an impasse that could spark U.S. retaliation.

Reagan administration officials contend that Japan has reaped enormous trade advantages by tapping freely into U.S. technology while keeping its own mostly to itself. The U.S. has been trying for months to reach a new accord that will redress that imbalance in the flow of commercially useful scientific information.

But disputes between the U.S. and Japanese negotiators are likely to prevent completion of a science and technology agreement to replace an existing accord that expires Jan. 31. Having failed to reach an agreement in time for Prime Minister Noboru Takeshita's visit here last week, the closed-door talks resumed this week. However, Japan's opposition to U.S. demands are so stiff it would be a "miracle" if the accord can be completed before the old one expires on Jan. 31, says a U.S. negotiator.

Federal Retaliation?

If the talks collapse, the U.S. could retaliate. A little-known 1986 statute authored by Sen. Robert Dole (R., Kan.) and Sen. John D. Rockefeller IV (D., W.Va.) authorizes federal laboratories to close their doors to Japanese researchers if Japan doesn't accord similar privileges to U.S. scientists. There are about 7,000 Japanese scientists and researchers working in the U.S., compared with only 500 American scientists working in Japan, estimates Mitchel Wallerstein of the National Research Council, which represents corporate and university science interests.

One reason for the disparity is that the bulk of U.S. basic research is done in open, usually public institutions such as national laboratories and universities. But, notes Sen. Rockefeller, most of Japan's best research is government funded but channeled to corporations.

"They've got the run of M.I.T. and Berkeley while our people can't get equal access to Japan's technology because most of its best stuff is in corporate laboratories," a U.S. official says.

To improve U.S. access to Japanese technology, the Japanese are being asked to publish more of their basic research in English, open up more research positions to Americans, and allow U.S. participation in product-development work financed by the Ministry of International Trade and Industry. "We don't have to define reciprocity in terms of the numbers of researchers in either country. We just want the principle recognized in the agreement," a U.S. negotiator says.

The Japanese government has promised to "increase our scientific cooperation with the U.S," and during his visit Mr. Takeshita offered the National Science Foundation a grant of $4.4 million to finance more trips to Japanese research centers by American scientists.

Addressing the Issues

But the U.S. wants a new scientific exchange agreement that addresses such issues as reciprocity, intellectual property rights and national security, while Japan prefers an accord that's as bland and toothless as the old one signed in 1980 by President Carter and the late Prime Minister Masayoshi Ohira.

In the area of patent secrecy, the U.S. wants the Japanese to acknowledge compliance with a 1956 bilateral accord designed to protect defense-related technology. Tokyo contends its current policies provide such protection though it resists making a public commitment for fear of antagonizing leftist opposition groups.

The U.S. also wants assurances that Japan won't unfairly commercialize jointly developed technology. Japan's access to a number of major projects, including a multinational space station and a supercollider to advance high-energy physics research, could depend on its willingness to satisfy such concerns, U.S. officials say.

Charles Owens, a National Science Foundation official involved in scientific exchanges with Japan, contends that Tokyo is making a serious effort to open corporate labs to U.S. researchers. "Our problem is finding American scientists who can read Japanese and handle the difficult cultural experience of working in Japan," he says.

U.S. scientists agree that language is an obstacle. But the problems go deeper. "Openness to foreign researchers isn't natural to Japan," says Richard Samuels, a professor who directs the Japan program at the Massachusetts Institute of Technology. "This has been true for 100 years."

Mr. Samuels worries that failure to reach an accord could interrupt important scientific cooperation. "There's no worse way for the countries to go than to stoke the fires of techno-nationalism," he says.

Swedish Current-Account Gap

Murex Corp. Covering All the Bases in Designing Novel Assays for AIDS

By Jay Asher

Screening tests for AIDS to date have been based on detecting the antibodies produced by the body in response to infection by the human immunodeficiency virus (HIV). Detection of these antibodies has been problematic for a variety of reasons. False positive results, which have been associated with anti-HLA antibodies, autoimmune sera, sera from cancer patients and frozen sera, may be due to a number of problems including technique difficulties. Improper sample handling and impurities within the test reagents. Other false positives may be associated with the test format and materials and the long incubation periods involved.

False negative results occur during the early stages of HIV infection if the test cannot detect early antibody response, one of the shortcomings of several available tests. Another source of false negative results is a decrease in antibody titer in advanced AIDS patients, where the immune system is so depleted that there is little antibody to detect.

The Western blot method is a preferred confirmatory test where a positive result for HIV antibody occurs. The Western blot involves antibody detection using a paper strip on which there is a characteristic pattern of separated viral antigens. This test, however, is expensive, takes a long time to perform and suffers from a lack of standardized results. A new generation of HIV tests is needed to overcome these difficulties.

Collaborates with Institute

In conjunction with a California research group, Murex Corp. (Norcross, Ga.) is working on a number of assays which will be directed to AIDS testing. The two-and-one-half year-old diagnostic firm has been securing an increasing variety of monoclonal antibodies to HIV through its own efforts as well as those of the scientists at the Institute for Cancer Research (ICR), an arm of the Medical Research Institute of the Pacific Presbyterian Medical Center, located in San Francisco, Calif.

A major result of this collaboration is the Murex Suds™ HIV test, a rapid, colorimetric immunoassay for the qualitative detection of antibody to HIV in serum and plasma samples. This enzyme immunoassay, which the company has not yet submitted to the FDA for approval, uses purified HIV antigens coupled to a solid phase via specific antibodies. The assay relies on antigen specific antibodies to increase the selectivity of the assay for early detection of antibody response to infection.

The Murex Suds™ HIV antibody test will be targeted at doctors' offices and clinics.

A human serum or plasma sample is incubated with the antigen coated solid phase for three minutes at room temperature. This mixture is transferred to a disposable Suds test cartridge designed for this application. The patented cartridge is a disk-shaped filtration device which incorporates a funnel opening for directing reagents onto the central area of a filter. Absorbent materials within the cartridge retain liquid used during the assay.

Unbound human sample is absorbed by the Suds test cartridge, while antibody specific for HIV binds to the solid phase and remains on the filter. An enzyme-labeled antibody to human immunoglobulin is added which binds to the HIV specific antibody. Unbound labeled antibody is removed by a wash solution into the Suds test cartridge. This step is followed by addition of a chromogenic substrate. If human antibody to HIV is present in the serum or plasma sample, a blue color is produced in the filter which can be detected visually in the bottom viewing area of the Suds test cartridge. Total test time is less than ten minutes.

Number of Assays

The HIV antibody test represents the latest in a number of assays being developed at Murex. Last June the company announced the signing of a marketing agreement with Syntex Corp. for distribution by Syntex of ten monoclonal antibody-based unit tests including hCG, Group A Strep, toxoplasmosis, rubella and other infectious diseases. The privately-held company is confident of developing a wide range of tests based in part on a supply of monoclonals to infectious diseases, which are being researched and produced by an affiliated company in Cambridge, England.

The relationship with ICR should provide Murex with access to a continuing source of materials for AIDS immunoassay development. ICR is researching new monoclonals to HIV for various Suds HIV assays, as well as other retroviruses and cancers.

Dr. John C. Klock, an AIDS researcher who works as a consultant for Murex, believes that as more drugs, such as AZT, show promise as a treatment for AIDS, there will be an increasing number of requests for AIDS testing in doctors' offices and clinics. Murex is targeting this market with its Suds assay.

Gwinnett firm leveloping test o detect AIDS

nda Aboff
it Daily News

ne midst of tranquil, wooded ndings in Peachtree Corners, sts are working diligently to o sensitive, accurate tests that tect the presence of an AIDS a matter of minutes.

scientists, who work for the Corp., a relatively new Gwin- technology firm, are well on ay to meeting their objective. his week, Murex was granted t for a diagnostic test device n accurately detect the pres- drugs, hormones and infec- seases in 10 minutes or less. her diagnostic tests currently le often can take several days t such conditions.

evice, called SUDS, for Single agnostic System, is a small, cartridge used to mix patient mples with reagents or bi- ered stances to quickly ect lood conditions.

y to Murex development e monoclonal antibody- chnology. These antibodies made cells that are capable gushing specific molecules, n can be used to diagnose conditions. The disease-spe- s are a fusion of the disease- antibodies and myeloma can-

has received the go-ahead Food and Drug Administra- iarket the SUDS device in n with specific diagnostic strep throat, pregnancy and to rubella and toxoplasmo- r causes of birth defects. e are expected to receive roval by the end of next

lossom, Murex vice presi- chief operating officer, said cartridge can be used to with any available Murex he SUDS system is the only ally available diagnostic in gents are sold separately test device, Hossom said. tly, the cartridge itself has ed shelf life.

rex tests are being mar- Murex through a venture lyntex Corp., a $1 billion tical company out of Palo

Murex's testing device

Alto, Calif. The SUDS device and tests are currently being marketed to hospitals, clinical laboratories and doctors' offices. Murex expects to begin selling them over-the-counter for home use in about a year.

Murex is currently developing other diagnostic tests that, when used with the SUDS device, can detect cancer, infectious diseases, herpes, acquired immune deficiency syndrome and other sexually-transmitted diseases, Hossom said.

The SUDS test device and diagnostic tests were developed by scientists and engineers at Murex's headquarters in Norcross. Two prototypes were developed before scientists successfully hit upon a device that accurately worked.

The test device also is manufactured at the firm's headquarters. Hossom said Murex is planning to produce at least 12 million devices a year.

SUDS was originally conceived on a cocktail napkin almost two years ago when Murex was first founded. Hossom said the road "from cocktail napkin to approval by the FDA" took only about 15 months, which is considered a remarkably short time to get through government red tape.

Julie Taylor of Murex adds reagents to

Three or four other larger compa payroll nies are working on similar products, year with but none have patents, Hossom said. facilities This is the privately held compa first patent. Twenty other patent ap plications, however, are pending. Murex

Murex was founded in March 1984 lab Research by Allen F. Campbell, who two years bridge, earlier had established a research Cancer group in Cambridge, England. Murex Murex em was initially formed to market the 130) of which bioengineering products Campbell's clerical research group developed, said Jared Kelsey, president and chief executive officer of Murex.

The company its total square footage The Norcross headquarters, which when it moves employs about 80 people, is located in ber. The new facility will include the Northwoods Business Park. The and development manufacturing areas, Kelsey said.

The Organization . . .

Murex Corporation
World Headquarters Norcross, Georgia (Greater Atlanta Area)

The **Murex** Corporate headquarters is situated in a beautifully landscaped business park, 15 miles north of the Atlanta business district in one of the fastest growing high technology areas in the nation. Atlanta provides the charm and elegance of the South and the sophistication of a major medical complex that includes the Centers for Disease Control (CDC). The Atlanta International Airport offers easy access to any city in the world. These are just a few of the aspects that position **Murex** as a dynamic force in international health care.

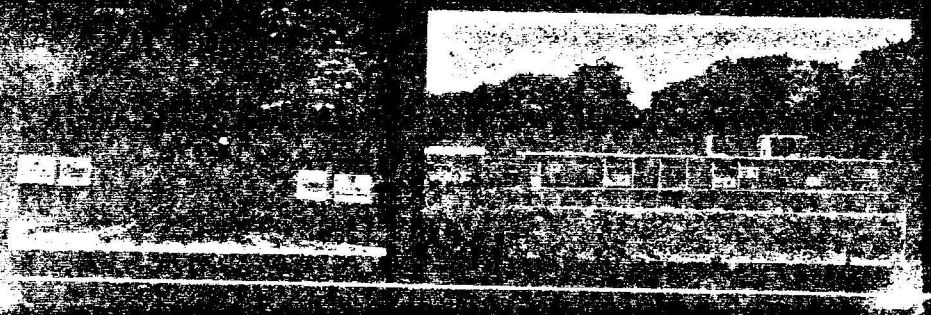
Murex Corporation, 3000 Northwoods Parkway, Norcross, Georgia 30071

Coral Sociedade Brasileira de Pesquisas e Desenvolvimento Ltda.
Sao Paulo, Brazil

Sao Paulo, Brazil, is a major center of infectious disease research in a city of approximately 10 million people. Due to the clinical nature of the work being conducted at Coral, the facilities have been conveniently located in a medical complex in the metropolitan area of Sao Paulo. Coral's clinical evaluation services provide a vast source of specimens for challenging the monoclonal antibodies.

Coral Sociedade Brasileira de Pesquisas e Desenvolvimento Ltda.,
Avenida Brigadeiro Faria Lima, 1620, 01452 Sao Paulo, SP, Brazil.

Coralab Research Cambridge, England



Coralab Research lies nestled in the beautiful English countryside of the Cambridge University Farm just off the major artery to town. In this serene setting over 40 scientists rapidly expand the already broad selection of monoclonal antibodies for **Murex**.

Coralab Research, Huntingdon Road Laboratories, Cambridge CB30DJ, United Kingdom.


nurex

murex™

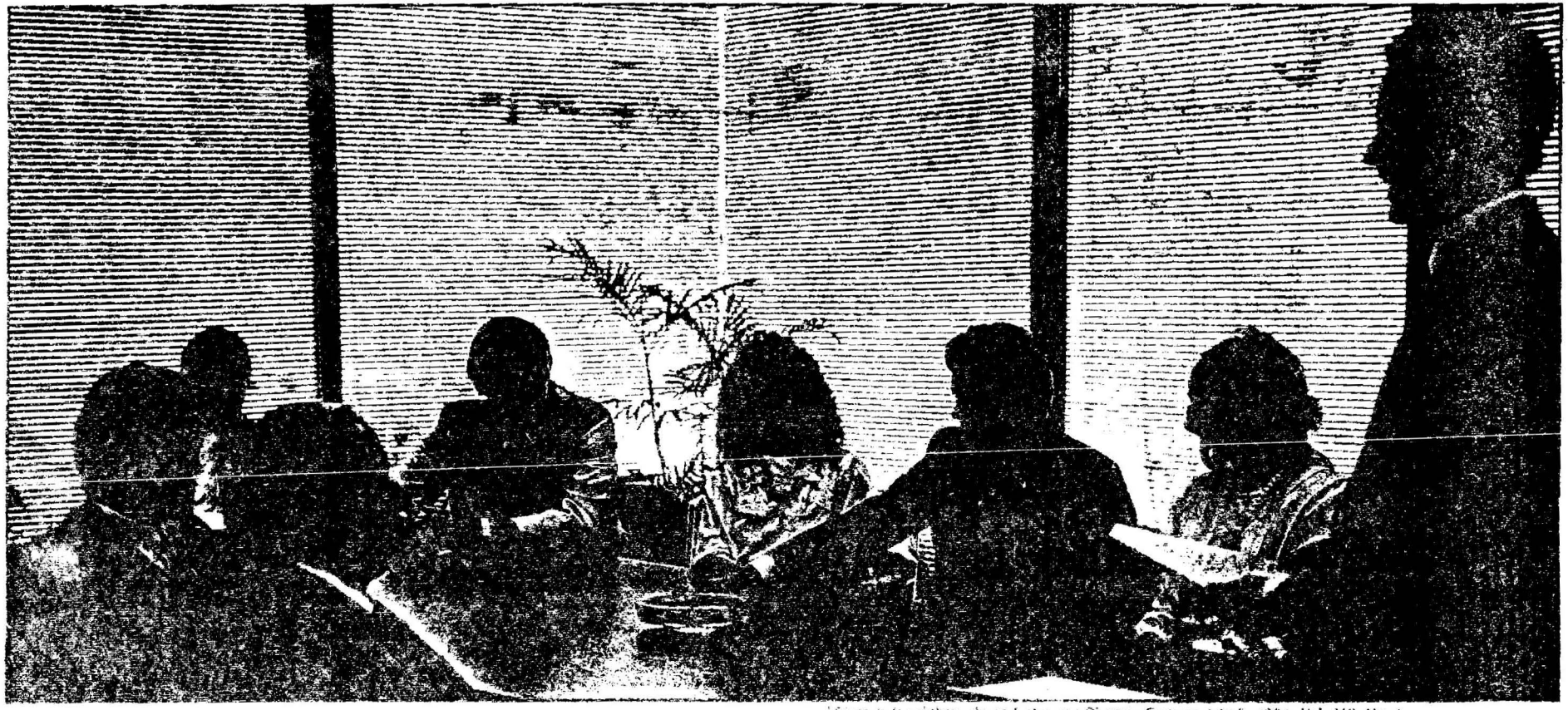
The Corporation . . .

Murex Corporation is a privately owned health care company, founded in 1984, to apply and commercialize the monoclonal technologies created over the past few years in Coral's research facilities. Unlike many "biotechnology" companies, **Murex** is a medical products company, dedicated to the creation of novel devices and instrumentation for medical diagnosis. The monoclonal antibodies offered for sale are by-products of the larger endeavor of employing these antibodies in important new products which will have a major impact on the medical world.

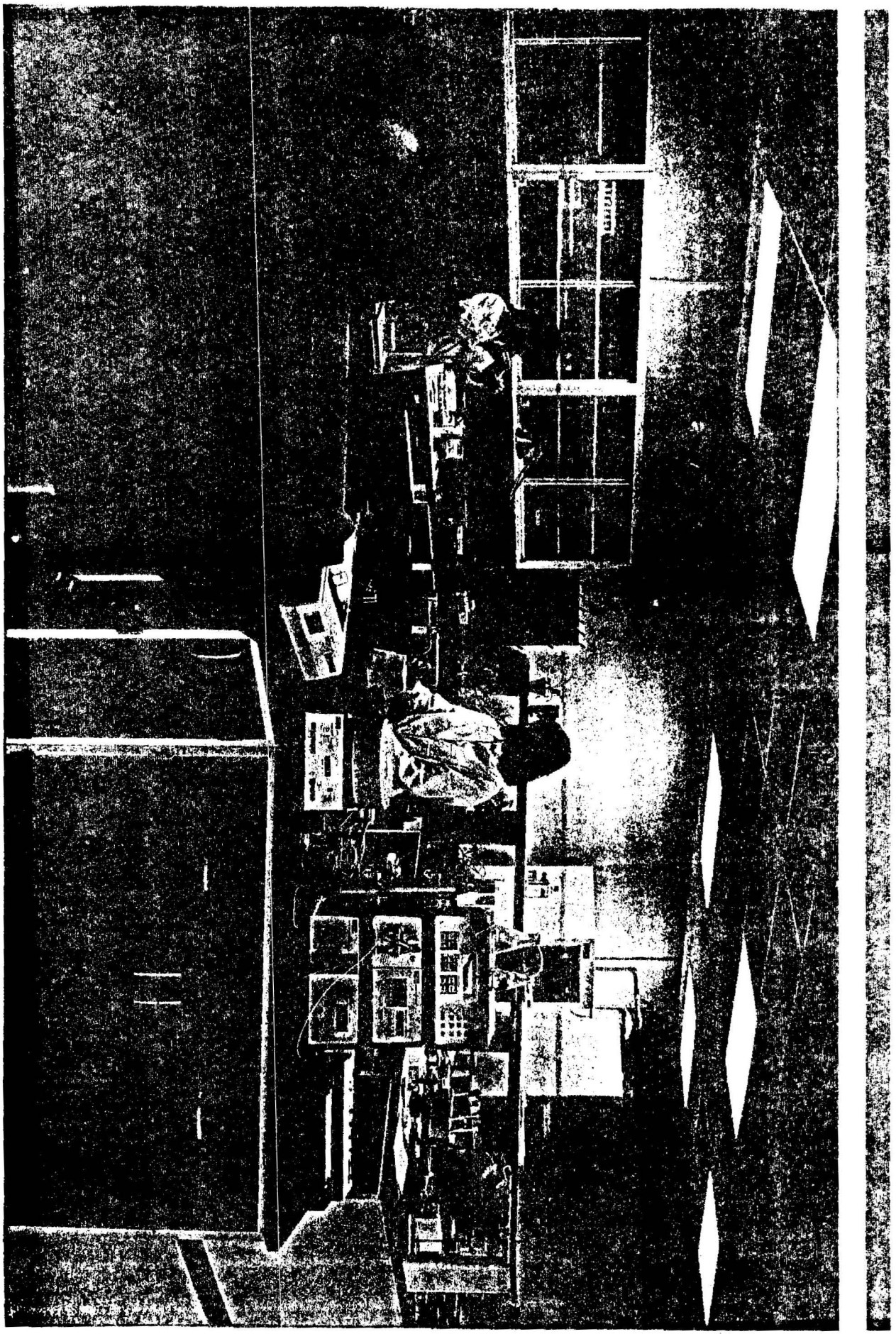
The Founders . . .

The founders of **Murex Corporation** are an international group of successful business executives and renowned scientists. Having previously organized and directed successful medical products companies, and representing a wealth of experience in biotechnology, infectious disease, engineering, marketing, and manufacturing of health care products, these founders now seek to address the needs of a $4 billion worldwide diagnostics market.[1]

Together they have assembled the components of a successful company . . . products . . . potential . . . and people.



[1] "Medical Breakthroughs in Infectious Disease, Fortune, July 9, 1984, Vol. 110, No. 1.



The Technology . . .

Monoclonal Antibodies

By 1990, many prevalent forms of cancer and infectious diseases will be detected and treated by agents linked to monoclonal antibodies . . . biological "guided missiles" which seek out and destroy invaders of the body such as viruses and bacteria. **Murex Corporation** provides a selection of monoclonal antibodies adopting the principle discovered in 1975 by Cesar Milstein and George Kohler while working at the Medical Research Council Laboratory of Molecular Biology, Cambridge, England. In 1984, the discovery of this principle brought them the greatest recognition in science . . . the Nobel Prize. **Murex** is a part of this tradition. Bruce Wright who directs Monoclonal Antibody Development at Coralab Research in Cambridge, England, was actively involved in the early development of monoclonal antibody technology at the Medical Research Council. **Murex** antibodies employ this prize-winning principle of cell fusion to produce monoclonal antibodies which will:

- cover the spectrum from bacteria and viruses to other disease markers
- utilize human, murine and other cell lines
- undergo extensive testing
- offer exquisite specificity

Diagnostic Test Systems

Murex technology focuses on the use of creative new immunodiagnostic test formats. At present, **Murex** research and development is concentrated [illegible]wo which will offer the choice of single-use simplicity or automation.

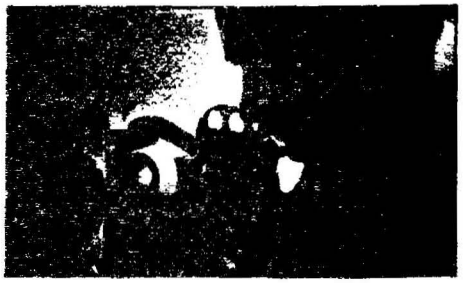
Single-Use Disposable System

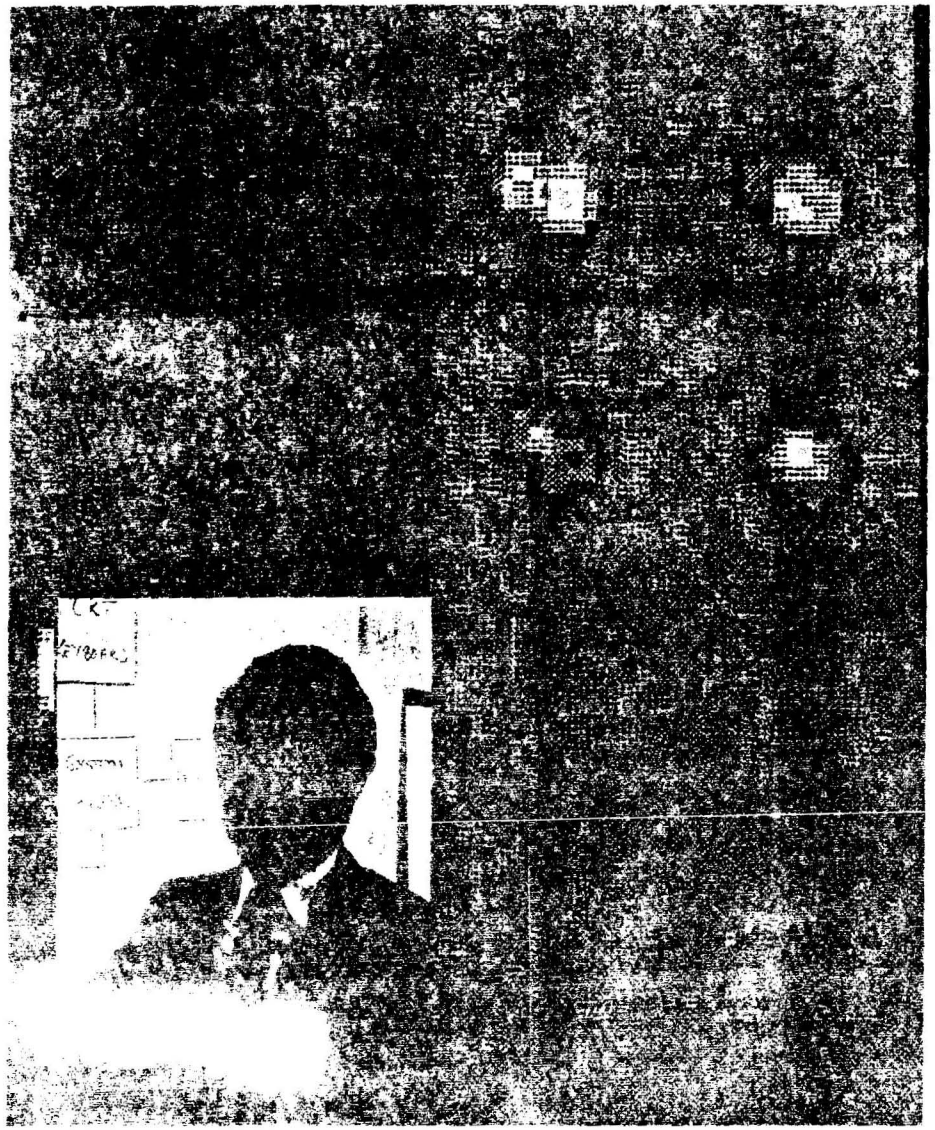
Intended for home, physician's office and hospital use, the **Murex** disposable system will provide nanogram sensitivity in a visually read test format that virtually eliminates opportunity for operator error. By employing a simple, low cost reading device, even greater sensitivity may be achieved. Quantitative assays which have previously required more expensive and time-consuming equipment may be within the grasp of the [illegible] physician. Test results can be available even before treatment is begun at a substantial savings.

Automation

For decades, man has strived to detect quantities of light energy measured in units called photons. This energy is emitted as electrons change energy levels during a chemical reaction. The ability to measure the energy released by two reacting molecules permits detection of invading antigens, such as viruses, bacteria and other substances, as they combine with labeled or tagged antibodies.

Murex engineers have concentrated their knowledge of electro-optics and software into a revolutionary instrument capable of detecting single immunological reactions between an individual antigen and antibody molecule. Incorporating the wide array of **Murex** monoclonal antibodies, the instrument will automatically detect such previously undetectable quantities of pathogenic substances present in blood, sputum, urine, stool and other body fluids, and provide a rapid and accurate diagnosis.





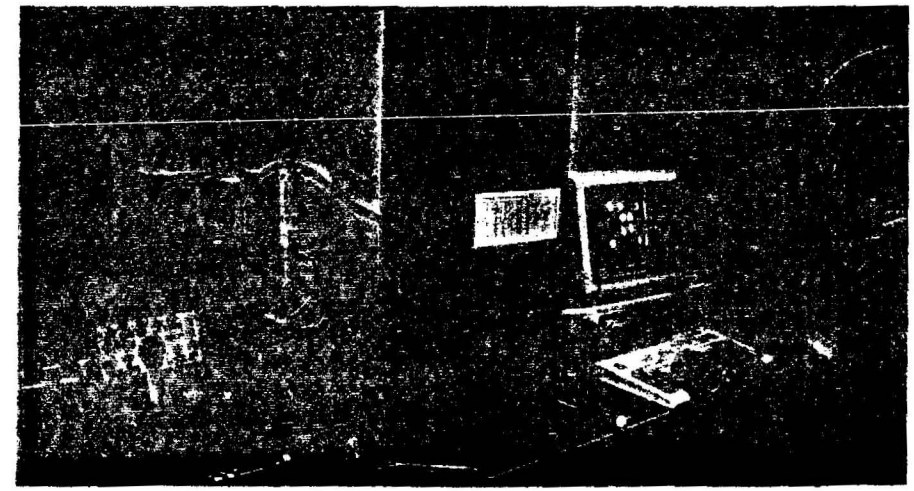
The Goal . . .

Medical science has for years strived to increase the sensitivity of the tests with which it detects disease. Through the years methods have been developed which detected milligrams (10^{-3}), then micrograms (10^{-6}) and nanograms (10^{-9}), and finally, picograms (10^{-12}, one trillionth of a gram) which is about where we are today. Each improvement achieving a thousandfold increase in sensitivity over the earlier generation; each step requiring some years of technical innovation.

In some laboratories today, the femtogram (10^{-15}, one-thousandth of a picogram) is being achieved under very carefully controlled conditions. Even the fabled attogram (10^{-18}) has been whispered as attainable in the foreseeable future.

Ironically, in terms of disease detection and diagnosis, these goals may still fall far short of ideal. In molecular terms, depending on the substance being detected, an attogram can be as many as 1000 molecules that must be present in a sample for it to be detected. Picogram sensitivity requires that 1 billion molecules be present in a specimen.

At **Murex**, our goal is the detection and identification of one molecule of any disease-related substance in a clinical specimen. Today, we are tantilizingly near that goal.



ORDER INFORMATION

When placing an order for Murex products, the following information must be furnished: product name, catalog number, vial size, product price, shipping and billing addresses and customer purchase order number.

Murex invites bulk orders, standing orders and contracts.

MAIL purchase orders to:

Murex Corporation
P.O. Box 2003
Norcross, GA 30091
Attention: Customer Service

TELEPHONE purchase orders to:

Customer Service
404-662-0660

Business Office hours: 8.30 A.M. - 5:00 P.M. E.S.T.

Written confirmation of all verbal orders is required.

TERMS AND CONDITIONS OF SALE FOR MUREX PRODUCTS

CONDITIONS FOR USE
The products labeled "For research use only and not for use in diagnostic procedures" should not be administered to humans or used for any diagnostic or drug purposes. These products are sold for research use only. Researchers intending to use these products procured herein for medical investigation on humans are solely responsible for such use and for compliance with the pertinent federal and state regulations, including those of the U.S. Food and Drug Administration.

PRICE
The purchase price shall be based on the price in effect at the time of sale. In addition, Buyer shall pay all governmental taxes, excises and other charges assessed against or incurred by Murex with respect to the sale, production or transportation of any goods delivered hereunder, except to the extent prohibited by apolicable law.

MINIMUM REQUIREMENT
Shipments for products totaling less than $100.00 will not be accepted without a service charge of $35.00. Reagents may be combined for minimum requirement purposes.

WARRANTY
All products are offered without warranty or guarantee of any kind (other than title) since the ultimate conditions of use are beyond Murex's control. It is understood that the Buyer will rely on its own tests to determine the suitability of [illegible] for its own particular purposes. THERE ARE NO EXPRESS [illegible] SET FORTH ABOVE, AND NO WARRANTIES (OTHER [illegible] UNDER THE UNIFORM COMMERCIAL CODE) [illegible] BY STATUTE OR OTHERWISE, INCLUDING WITHOUT [illegible] MERCHANTABILITY AND WARRANTY OF FITNESS [illegible] PURPOSE.

The Buyer's receipt of any goods delivered hereunder shall be an unqualified acceptance of, and a waiver by Buyer of any and all claims with respect to such goods unless Buyer gives Murex written notice of claim within fifteen (15) days after such receipt.

Murex does not, by reason of the sale and delivery of goods hereunder, grant Buyer any right to use the goods in the practice of any process or in combination with other materials where such process or combination is covered by a patent of Murex. Murex does not suggest, recommend, warrant or represent that the goods delivered hereunder can be used in the practice of any patented invention of others without a license. Buyer assumes all responsibility for determining whether relevant patents exist covering any contemplated use by Buyer of the goods delivered hereunder.

DELIVERY TERMS
Buyer agrees to pay freight on any shipment less than $1000.00. Murex agrees to pay freight on any shipment above $1000.00. The value of reagents may be combined to meet the $1000.00 minimum shipping requirement. Murex maintains the right to select the carrier for all prepaid shipments.

PAYMENT TERMS
Net 30 days from date of invoice; interest charge assessed at 1½% of unpaid balance.

GOVERNING LAW
The laws of the State of Georgia shall govern this sale.

ADJUSTMENTS OR CANCELLATIONS
Adjustments to or cancellation of an order must be made at least 15 days prior to scheduled arrival date, and must be made through your Customer Service Representative.

RETURNS
No returns will be accepted without proper written authorization from Murex Corporation.

If returns are made due to Murex error, no charge will be assessed against the Buyer. A return of 20% will be assessed in connection with returns of merchandise originally shipped as ordered. No returns will be authorized with respect to opened reagents.

Cat. No.	Unit	Product Description	Specificity	Clone	Murex Microbial Test Systems	Sub Class	Form of Antibody	Unit Price
01-002-3	0.5mg	E. coli 02	NCTC 11100 & 9002	1002/224	EC. 116. SL. SG. KI. 2. 3. PI. 2. 3. 4. ENTI. 2. 3. 4	IgG3	Purified	
01-002-4	0.5mg	E. coli 02	NCTC 11100 & 9002	1002/224	EC. 116. SL. SG. KI. 2. 3. PI. 2. 3. 4. ENTI. 2. 3. 4	IgG3	Conjugate	
01-003-3	0.5mg	E. coli 03	NCTC 9003	144/75	116. EC1-7. PI. KI. SL. SG. W. CAM. SERRI. ENTI	IgG1	Purified	£10.00
01-005-3	0.5mg	E. coli 05	NCTC 9005	23/266	EC. SL. SG. KI. 2. 3. PI. 2. 3. 4. ENTI. 2. 3. 4	IgG3	Purified	£10.00
01-005-4	0.5mg	E. coli 05	NCTC 9005	23/266	EC. SL. SG. KI. 2. 3. PI. 2. 3. 4. ENTI. 2. 3. 4	IgG3	Conjugate	£155.00

[illegible] INFORMATION

[illegible] the product of the fusion of the mouse [illegible] [illegible] mice, unless specifically [illegible] otherwise.

[illegible] TYPES

[illegible] are prepared from ascites or tissue culture supernatants. [illegible] antibodies are purified from ascites by either ammonium sulfate precipitation [illegible] by DEAE [illegible] chromatography or protein A Sepharose affinity chromatography. [illegible] antibodies are purified from supernatants by either protein [illegible] affinity chromatography or a combination of SP-Sephadex [illegible] ammonium sulfate precipitation and DEAE cellulose [illegible] are purified by other chromatography methods.

[illegible] prepared from cell culture fluid by centrifugation [illegible] through glass wool and then high

[illegible] in the catalog was [illegible] (EIA) testing [illegible] Systems [illegible] It is not warranted [illegible] in the [illegible] system [illegible] to excessive

[illegible] antibodies are supplied either in 10 mM TRIS + 1 mM [illegible] pH 7.5 containing 1% BSA as stabilizer or in 1:1 PBS/glycerol.

[illegible] alkaline phosphatase conjugated antisera [illegible] as a preservative. Sodium azide may react with lead [illegible] to produce metal azides which are explosive [illegible] [illegible] waste disposal.

GENERAL COUNSEL OF THE
UNITED STATES DEPARTMENT OF COMMERCE
Washington, D C 20230

28 MAY 1986

MEMORANDUM FOR: Douglas A. Riggs
General Counsel

THRU: Robert H. Brumley
Deputy General Counsel

FROM: Robert B. Ellert
Chief Counsel for Economic Affairs

SUBJECT: NTIS Involvement in AIDS Patent Dispute

This memorandum is to further advise you of the involvement of the National Technical Information Service (NTIS) in the dispute between the National Institutes of Health (NIH) and the French Institute Pasteur (Pasteur) over which of the latter two organizations was the first to isolate the viral agent of AIDS. This memorandum particularly reports upon developments that have occurred since my April 3 memorandum to Mr. Brumley. (Tab A)

Background of the Dispute

In May of 1985 a United States patent was issued to NIH for an AIDS test kit that permits the accurate diagnosis of the AIDS virus, and that may also be used to screen blood banks to prevent contaminated blood from being used in transfusions. The validity of that patent, however, is now under serious challenge by Pasteur. A competing patent application was filed by Pasteur in the U.S. Patent Office in November of 1983, fully six months before the NIH application was filed in April of 1984. No patent, however, has issued upon the French application. The French argue that their patent application should have received priority in the U.S. Patent Office, and further, that much of the NIH research is based upon a live AIDS virus that Pasteur claims that it provided to NIH, and that NIH improperly appropriated for its own use. In addition, Pasteur claims that its test kit is superior to the NIH kits, producing substantially fewer false positive test results than the NIH kits.

Pasteur has sought remedies in several fora, including the courts and the U.S. Patent Office. It has brought suit against NIH in the Claims Court, alleging that it, and not NIH, was the first to isolate the AIDS virus, and further, that NIH breached contractual

agreements between NIH and Pasteur by illegally converting that virus to NIH's use in patent applications. (Institute Pasteur v. United States, Claims Court No. 730-85c.) Pasteur seeks, among other things, an order that it was the first to isolate the viral agent of AIDS, and that it is entitled to all profits and royalties from sales or distribution of AIDS test kits manufactured and sold under the NIH patent. Pasteur has also pursued its challenge in the U.S. Patent and Trademark Office, where it has instituted an interference proceeding. The Patent and Trademark Office decided to allow formal proceedings on the two patents on April 30, and has granted Pasteur "senior party" status. This effectively shifts the burden of proof to NIH in the interference proceeding. At stake in these actions is perhaps $3-5 million in annual royalty payments from the patent, plus substantial international recognition.

These actions are being defended by the Department of Justice, with the support of HHS. While HHS has had a number of discussions with attorneys for Pasteur in an attempt to resolve these disputes short of litigation, in late April these negotiations broke down. HHS has requested OMB for approval to fund its litigation expenses out of the royalties received by NTIS under the AIDS licenses. This use would be consistent with the interagency agreement between HHS and NTIS which provides that NTIS may use royalty payments to offset costs incurred for the filing and prosecution of patent applications. (Tab B).

NTIS Involvement

NTIS and the Department are involved in actions stemming from the fact that NIH has transferred custody of the patent in question to the Secretary of Commerce, and the NTIS Patent Licensing Program has granted nonexclusive licenses to use the NIH patent to five U.S. firms. Pasteur has established a license to sell AIDS test kits in the United States with Genetic Systems, Inc., a subsidiary of Bristol-Myers. The test kits, which gained FDA approval on February 18 and now being marketed by Genetic Systems, are alleged BY NIH and some of the NTIS licensees, to infringe upon the NIH patent.

The NTIS license agreement with its licensees deals with infringement as follows:

> "6.1 Licensee shall notify NTIS promptly in writing of any infringement of patent rights which becomes known to licensee.
>
> "6.2 In the event that NTIS determines that a substantial infringement of patent rights exists, which determination shall be made by written notice to

licensee, NTIS shall take prompt action to attempt to eliminate that substantial infringement. Licensee shall, at the request of NTIS, cooperate fully in gathering information concerning whether an infringement of patent rights constitutes a substantial infringement for the purpose of this article. NTIS shall notify licensee (within thirty (30) days following licensee's notice under paragraph 6.1), in writing, of its determination that a substantial infringement of patent rights exists and that NTIS will attempt to eliminate that substantial infringement.

"6.3 Should NTIS be unsuccessful in eliminating the substantial infringement within ninety (90) days following licensee's notice under paragraph 6.2, NTIS agrees to recommend to the appropriate United States Government authorities that an infringement action based on patent rights be initiated. Licensee shall at NTIS' request cooperate in every respect including making available to NTIS records, information, evidence, and testimony by employees of licensee relevant to the substantial infringement of the licensed patent rights.

"6.4 If, after twelve (12) months from the date of the written decision by NTIS to attempt to eliminate infringement of the patent rights, NTIS has not eliminated the infringement of patent rights or if the United States Government has not initiated an infringement suit, licensee may cease payment of royalties due hereunder resulting from sales of licensed products. When such infringement has been eliminated, or an appropriate infringement suit has been initiated, the obligation to pay the royalties shall resume, royalties being due only from the date the infringement is eliminated or from the date an infringement action is initiated".

To date, we have had notice of a possible infringement from three of the five NTIS licensees. (Tab C.) Each has requested that we initiate action under section 6.2 of the license to abate the infringement. Additionally, Dr. Donald J. Macdonald, Acting Assistant Secretary for Health at HHS and the official with policy oversight over NIH, has requested that the Director of NTIS initiate a patent infringement action against Genetic Systems. (Tab D.) Dr. Macdonald's letter stated that the NIH patent counsel was of the opinion that there was an infringement, and also included an opinion prepared by John S. Roberts, the private attorney who had filed the original NIH patent application, stating that the Genetic Systems test kit did indeed infringe upon the NIH patent. An independent analysis by Eugene Pawlikowski, a patent attorney in my office, also concluded that there was an infringement.

the patent rights, NTIS has not eliminated the

Based on the available record, in my view there is clearly an infringement of the NIH patent by Genetic Systems. However, under section 6.2 of the license agreements the infringement must be "substantial". The same section also authorizes NTIS to request its licensees to gather information as to "whether an infringement of patent rights constitutes a substantial infringement" warranting an infringement action. The record now available shows only one bona-fide sale: to the Health Care Information Center of New York. While evidence of infringement, it does not support a determination of a "substantial" infringement. We have asked NTIS to request its licensees, under section 6.2, to gather more information on the extent of market activity and sales by Genetic Systems. NTIS is reserving its judgement as to whether or not there is a "substantial" infringement until more evidence is received.

Assuming additional evidence of sales by Genetic Systems is received, and a "substantial" infringement is established, we should consider proceeding with the tentative decision that was made on May 7 in a meeting I attended with attorneys from NIH and the Department of Justice. That is, prior to initiating litigation an attempt should be made to cure the "substantial" infringement by offering Genetic Systems either a license or sublicense to practice the NIH invention. The following steps would be required in such an eventuality:

(a) Determination by the Director of NTIS under section 6.2 of the licenses that there is a "substantial" infringement.

(b) Attempt by NTIS to cure the "substantial" infringement by suggesting that Genetic Systems obtain a sublicense from one of the five NIH licensees. NTIS has advised that at least one licensee would grant a sublicense. Such a sublicense would offer Genetic Systems substantially less favorable terms than would a direct license. HHS, however, opposes offering Genetic Systems a sixth direct license to practice the NIH invention, as such a license may be used as part of a settlement agreement with Pasteur.

(c) Notice to the five licensees that the Director of NTIS has determined under section 6.2 that a "significant" infringement has occurred.

If Genetic Systems refuses to accept a sublicense from one of the five licensees, under the terms of the licenses NTIS would be obligated to recommend to the "appropriate United States Government authorities", in this case the Department's Office of General Counsel, that litigation be initiated to end the infringement. We would then have one year to consider the pros and cons of requesting that the Department of Justice file an

infringement suit, before the licensees were relieved of the obligation to pay further royalties to NTIS. In that time, the status of the interference proceeding might become more clear. Further, I am told by Darrel J. Grinstead, Assistant General Counsel at HHS, that Pasteur may be seeking to reopen discussions for a settlement. This situation too might be clarified over the next year.

The main advantages to eventually seeking an infringement action is to protect the royalty stream of income to the U.S. Treasury generated by the licenses, and to maintain the integrity of the NTIS Patent Licensing Program. On the other hand, there is the disadvantage of possible criticism that the Department is attacking the sale of what might be a superior AIDS test kit. Given the present uncertainties in this matter, a decision on litigation should not be made at this time.

Analysis of Proposed AIDS agreement

PROVISION

"...have agreed to integrate their rights or claims to royalties...they have agreed to place these royalties into a foundation..."

Current Law This would not be allowed. All royalties in excess of the costs of licensing must be returned to treasury.

H.R. 3773 The NIH could make this agreement, which would apply to the royalties received after payment of the inventor's share, if they also get HEW agreement to return the full amount after the inventor's share to NIH rather than drain some off for other laboratories.

PROVISION

"...to be established for furthering research in prevention and treatment of AIDS"

Current Law This would not be allowed.

H.R. 3773 This would be allowed under 12 (b) (2) (A) so long as the AIDS research is consistent with the agency mission.

PROVISION

"The IP and NIH/NCI will each receive annually from the FAAF, for continued support of research, and amount..."

Current Law This would not happen since the US share of the royalties would not get to the FAAF in the first place.

H.R. 3773 This would be allowed under 11 (a).

PROVISION

"...the FAAF will own both patents."

Current Law There is no authority for agencies to assign ownership of an invention to other parties.

H.R. 3773 The bill does not provide authority to assign ownership of existing inventions, but since there is authority to pool the royalties, this should be adequate.

NOTE

Nothing in the proposed agreement discusses allocation of rights to future inventions under the various combinations of situations that might occur.

United States Senate

COMMITTEE ON THE JUDICIARY
WASHINGTON, DC 20510

July 29, 1986

Norm Latker, Esq.
OPTI, Room 4837
Department of Commerce
14th and Constitution Avenue, N.W.
Washington, D.C. 20230

Dear Norm:

Per our conversation of today, enclosed please find a copy of a proposal for a settlement framework for the Institut Pasteur case. This is now under discussion between HHS and the Institut Pasteur, with the full encouragement of this subcommittee.

Because of your familiarity with the policy issues presented by the proposal to direct royalties for the AIDS diagnostic test kits to a Franco-American AIDS foundation, I would appreciate any thoughts you may have about the best way to harmonize this settlement with existing policy developments, including the pending legislation on inventions by federal employees. I would also be interested in your advice on the need, if any, for specific legislative authorization of the disposition of royalties contemplated by this settlement proposal.

Sincerely,

Steven J. Metalitz
Chief Counsel and Staff Director

SJM:vs
Enclosure