Biomedical aspects of HIV and AIDS

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IN 1981 reports of immunodeficiency associated with the development of pneumocystis carinii pneumonia in homosexual men on both the East and West coasts of the United States brought to the world's attention the beginning of what is now referred to as the AIDS epidemic[1]. Previously healthy men had, for no apparent reason, acquired an immune deficiency state that was so serious that they could no longer protect themselves from usually readily dismissed organisms such as pneumocystis carinii. Physicians in the Western world had not encountered such a syndrome before. Because the new disease initially affected homosexual men, concepts of a "gay plague" became popular and much effort was expended on looking for unique features of the homosexual life-style that might destroy immunological competence. Any retrospective review must realize that no knowledge of the epidemic of AIDS that had been sweeping through Africa for at least thirty years was available to help clinicians understand the disease they were now seeing in the West.

With the discovery that the human immunodeficiency virus (HIV) was responsible for destroying the immune system and producing AIDS[2] the pandemic nature of the problem became clear. At this writing, the World Health Organization's Global Programme on AIDS estimates that some eighteen million adults and one-and-a-half million children are infected with HIV and that between 5000 and 10,000 individuals are infected every 24 hours[3]. In 1994 more than three million adults became infected with the virus. The epidemic continues to spread at ever faster rates in many developing countries, with the epicentre of the epidemic moving inexorably from Africa to Asia. Already, India may have more individuals infected with HIV than any other country. This review will examine the biomedical aspects of HIV infection that are producing much individual human suffering and socioeconomic disruption in many countries.

Virology

Only in the last fifteen years have investigators realized that members of the retroviral family of viruses are capable of producing disease in humans[4]. Prior to that time the medical community had known that these viruses could produce disturbances of the immune and nervous systems of animals, with the immunodeficiency produced often being associated with some form of malignancy. A competent immune system is necessary to protect us from certain forms of cancer. We now know that one distinct branch of the retroviral family can produce leukaemia in humans while another branch of the family contains two viruses, HIV-1 and HIV-2, that can destroy the most important, indeed pivotal, cells within the immune system: CD4 lymphocytes. These cells are members of the T cell family of lymphocytes produced in the thymus gland. While the destruction of sufficient numbers of CD4 lymphocytes to produce immunodeficiency takes a number of years (there are approximately six hundred billion of such cells available to an adult), a threshold is reached when there are simply too few of these cells available to protect an individual from invading microorganism and the clinical syndrome AIDS develops.

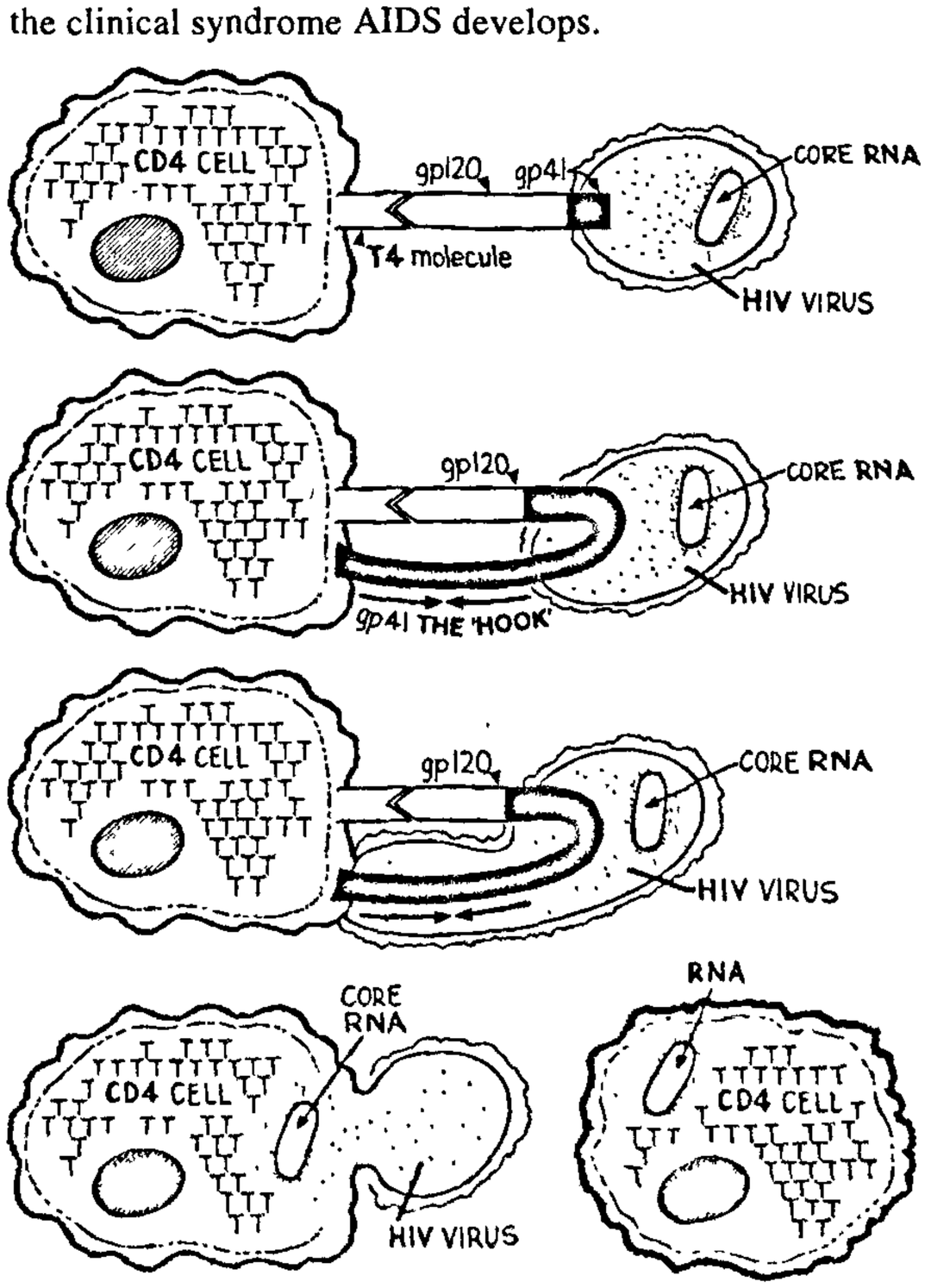

Figure 1. The binding and fusing of HIV to CD4 cells

Retroviruses are composed of RNA and to reproduce must insert their genetic information into the DNA of the nucleus of the cell they invade. To accomplish this, they carry with them a unique enzyme, reverse transcriptase, that once activated inside a parasitized cell can translate RNA into DNA. It is this 'turning' from RNA into DNA that gives the family its name ('retro'). The virus is composed of an envelope from which protrude unique three-dimensional structures contained within a large molecule (molecular weight (MW) 120,000 Da) (Figure 1). This protein, gpl20, allows HIV to bind with exquisite specificity to a molecule present on the surface of CD4 thymus-derived lymphocytes. These cells are involved in initial recognition of 'foreignness' i.e. antigenic determinants which are recognized in the form of small amino acid sequences. CD4 lymphocytes do not react to self-determinants but do react to foreignness, initiating a cascade of immunological responses that ultimately lead to the elimination of non-self protein[5]. Currently, we recognize two distinct forms of CD4 lymphocyte. THE cells can stimulate cells referred to as CD8 lymphocytes (also derived from the thymus gland) to destroy directly or help produce an inflammatory response to viral, fungal and parasitic invaders. The second type of CD4 lymphocyte (TH2 cells) activate bone-marrow-derived lymphocytes, 'B cells', to produce antibodies that are essential for the elimination of extracellular bacteria[6].

Because CD4 lymphocytes play a pivotal role in initiating an effective immune response, the invasion and destruction of these cells by HIV-1 and HIV-2 cripples the entire immune apparatus.

As mouse lymphocytes into which the genes for the CD4 molecule have been inserted cannot be infected with HIV-1 or HIV-2, despite the resulting expression of the CD4 molecule on their surface, researchers have long argued that a second receptor is necessary for HIV invasion of lymphocytes. Recently scientists at the Institute Pasteur in Paris have suggested that the second molecule is CD26, a widely distributed protein present on many cells including CD4 lymphocytes[7].

A transmembrane protein in the envelope of HIV-1 and HIV-2 referred to as gp41, as it has a molecular weight of 41,000 Da, is capable of fusing the envelope of HIV with the membrane of a CD4 lymphocyte that has bound the viral receptor molecule gpl20. With the fusion of the two membranes, the core of HIV finds itself in the cytoplasm of the attacked CD4 lymphocyte. Activation of reverse transcriptase, insertion of proviral DNA and the subsequent replication of HIV within the T cell leads to both replication of the virus and the destruction of that particular T lymphocyte[8].

An addition to specific genes that code for the production of the viral envelope or the RNA sequences needed for replication and reverse transcriptase, HIV-1 and HIV-2 contain a series of genes that produce regulatory proteins. While some of the products of these genes may be directly toxic to CD4 lymphocytes, their major role is to activate and control the replication of HIV in an infected cell. These regulatory genes are potential targets for therapeutic intervention strategies. Thus, HIV has a unique capability to invade and destroy thymus-derived CD4 lymphocytes, thereby producing, over an extended period of time, such a gross immune deficiency that infected individuals can no longer limit the replication of HIV or respond effectively to other infectious agents that take the 'opportunity' presented to them to produce serious clinical disease.

Immunology

Infection with HIV produces, within six weeks, an effective immune response that limits the initial burst of viral replication. An effective response involves the production of neutralizing antibodies but, more importantly, the activation of CD8 lymphocytes that can destroy virus-infected cells and/or secrete an as yet uncharacterized lymphokine that inhibits viral replication. Lymphokines are hormone-like chemicals produced by activated lymphocytes to carry activation and suppressive signals from one cell involved in an immune response to another. Patients infected with HIV, though infectious, remain well for a number of years after infection occurs as a result of this immune response. No evidence exists to date to suggest that the immune response of anybody infected with HIV has successfully eliminated the virus or produced an immunological response that would make one confident that AIDS will never develop. While 67% of patients infected with HIV will have developed AIDS within ten years of infection, those that stay well longer are currently being investigated to determine if they make a more effective immune response to HIV than those who are ill at the end of ten years of infection.

Within a few weeks of infection the number of CD8 lymphocytes present in the circulation of people infected with HIV increases markedly. Most of the CD8 lymphocytes belong to that subclass of thymus-derived lymphocytes that are capable of attacking virus-infected cells. The total number of circulating CD4 lymphocytes usually undergoes an initial and significant fall around the time of seroconversion before stabilizing for many years. The replication of the virus in the first few weeks of infection is associated with permanent destruction of a significant number of CD4 lymphocytes.

HIV is cleared from the plasma of an infected individual at the time of seroconversion as antibodies remove the virus present and CD8 lymphocytes minimize further production. From then on, finding viral proteins in serum or indeed in peripheral blood lymphocytes becomes difficult. Thus, while it is possible throughout the course

of an active infection with the hepatitis B virus to find viral protein in the blood stream, we rely on the presence of antibodies to HIV to diagnose the presence of that virus. We now know that most of the infected CD4 lymphocytes pool rapidly within the lymphoid tissue of infected individuals. Lymphoid tissue is organized into nodes or glands that are scattered around the body at critical sites. The immune system prefers to fight its immunological battles in these highly organized centres. It is probable, but not established, that this pooling is associated with activation of signals that attract HIV-infected cells into lymphoid tissue. Certainly, a significant number of patients rapidly develop lymphadenopathy (swollen lymph glands) and recent studies strongly suggest that the destruction of most of CD4 lymphocytes by HIV-induced mechanisms occurs in lymphoid tissue rather than in the circulation[9].

Ultimately, the immune system's effectiveness in controlling HIV is compromised because of the ongoing destruction of more and more CD4 lymphocytes. Some four to five years after initial infection, some selective process, as yet uncharacterized, commonly seems to destroy THE CD4 lymphocytes more rapidly than those capable of stimulating an antibody response (TH2 cells). Thus, although the number of CD4 lymphocytes in the circulation may remain relatively stable, the number of cells capable of stimulating an effective cell-mediated immune (CD8) response continues to fall. This dramatic change in the immunobiology of the natural history of the disease is not associated with changes in an infected individual's clinical state.

Numerous reasons are known to be associated with the virus's capacity to evade an effective immune response and ultimately, in most cases studied, destroy effective immunity. HIV can destroy cells that are not directly

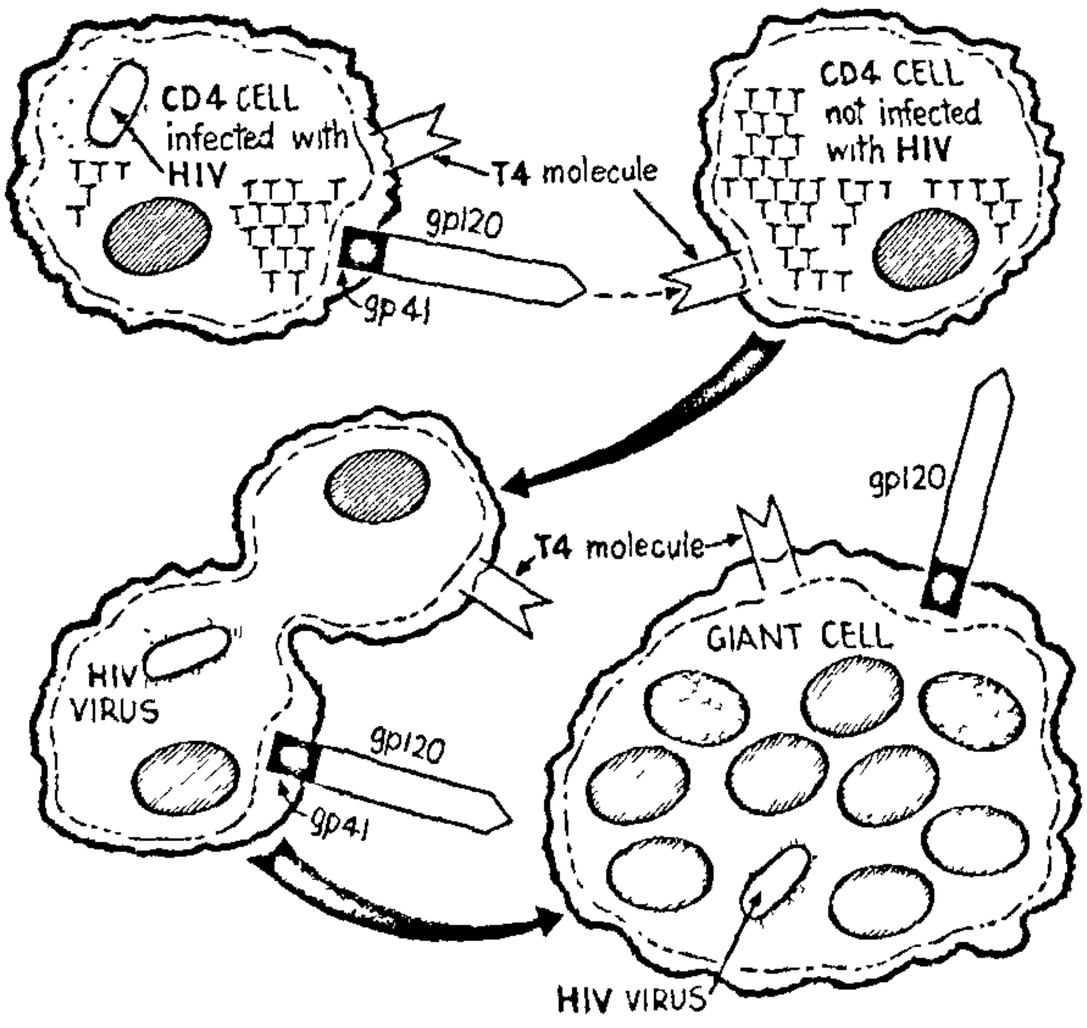

Figure 2. Two indirect methods used by HIV to kill CD4 cells.

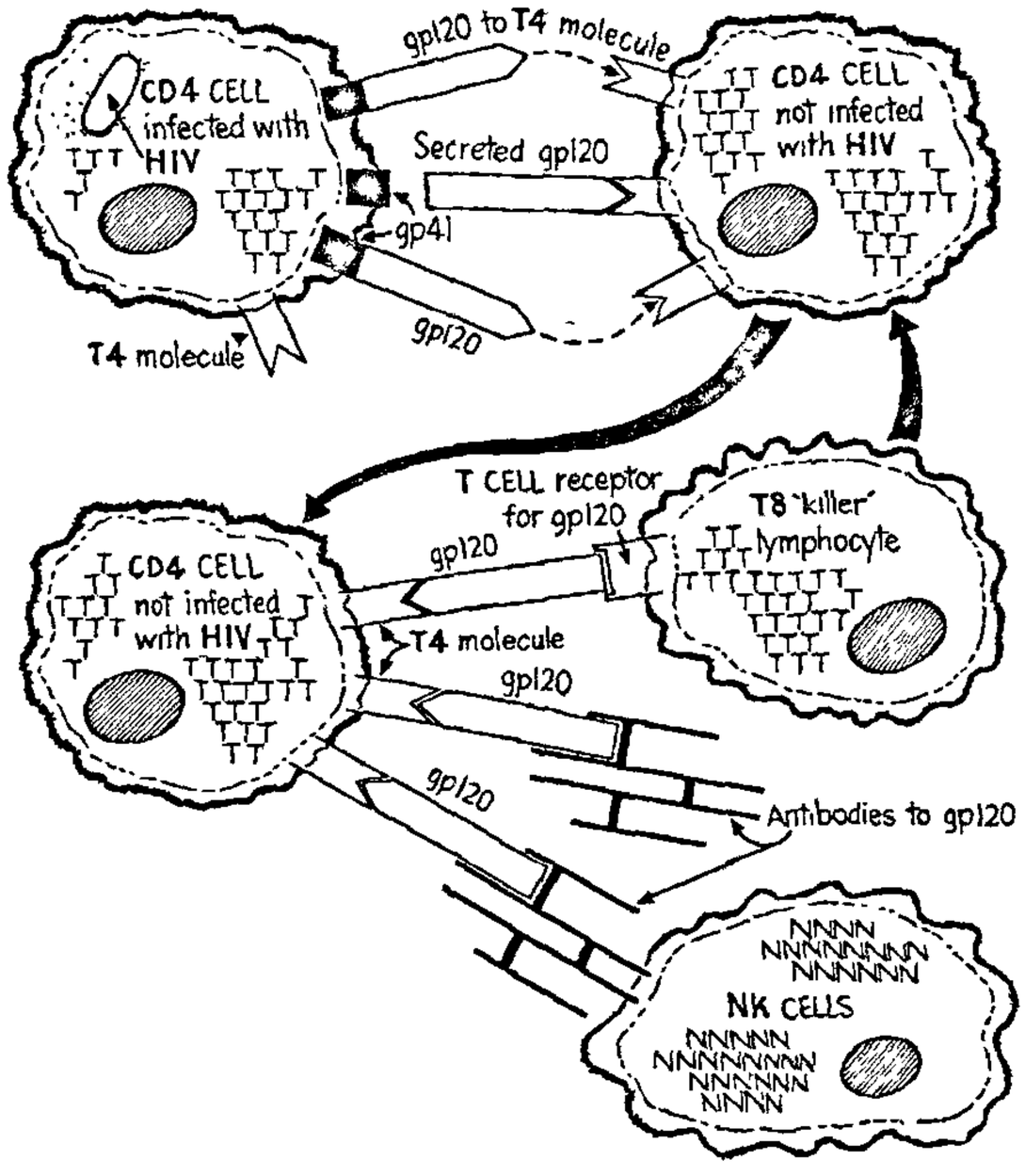

Figure 3. Normal CD4 (T4) cell destroyed by antibodies, NK or T8 cells because it bound secreted gp120

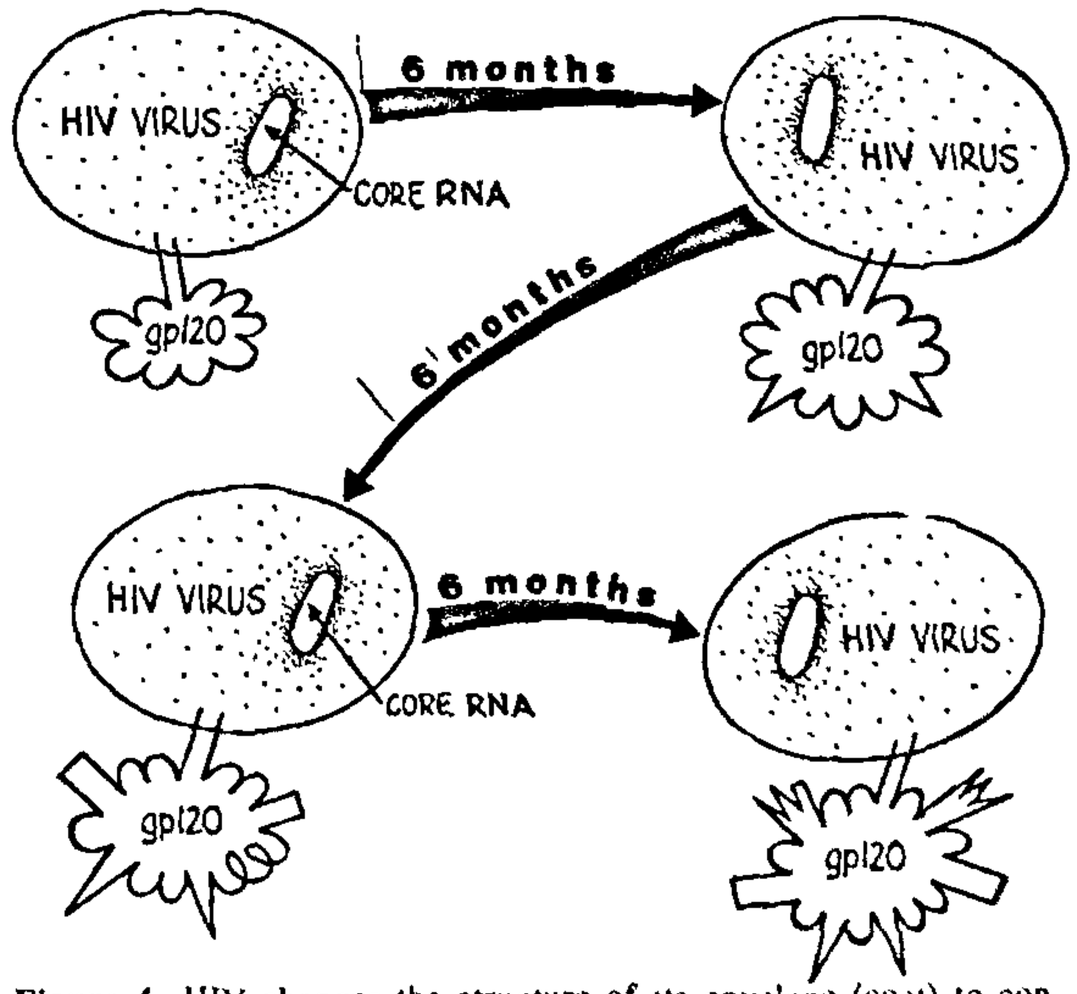

Figure 4. HIV changes the structure of its envelope (coat) to confuse attacking T8 cells.

infected with HIV at all (Figure 2). In cells infected with HIV in which the virus is actually replicating, envelope proteins (gp120 and gp41) are expressed on the surface of the cell, thus enabling an infected cell to bind the CD4 molecule of passing uninfected lymphocytes (Figure 3). As these envelope proteins contain the fusigenic moiety (gp41), giant cells (or syncitia) are formed. gp41 fuses the outer membrane of the T cell to the coat of the virus. At the

point of contact the contiguous membranes dissolve and in this way the core of the virus finds itself inside the CD4 lymphocyte. By the formation of these giant cells as infected cells trap uninfected cells it is possible for one infected cell to kill five hundred uninfected cells.

The secretion from infected cells of envelope proteins that will bind to CD4 molecules present on uninfected cells sets these unfortunate cells up as targets for cellular and humoral responses generated against envelope proteins. In addition to these strategies HIV-1 and HIV-2 are capable of generating considerable antigenic diversity among their envelope proteins. This constant antigenic 'drift' interferes with the perpetuation of an effective secondary immune response and requires the constant reactivation of primary immune responses to handle the variations presented to the immune system.

Clinical considerations

Infected CD4 lymphocytes, rather than free virions (elementary virus particles), are the important vector for HIV in human to human transmission. HIV-containing CD4 lymphocytes in sufficient concentration to make a biological fluid infectious are found in breast milk, blood and the sexual secretions of males and females. HIV can thus spread from an infected to an uninfected individual during vaginal or anal intercourse if infected blood from one individual enters the body of another and occasionally during breast feeding, particularly if a mother is in an early or very late stage of infection. At both extremes the replication of virus is most rapid and the concentration of virus in biological fluids reaches highest levels.

Within four to six weeks of infection, a seroconverting illness is noted by many, but these flu-like symptoms may not be particularly dramatic and are frequently not remembered by infected individuals. The symptomatology, which may occasionally feature significant pain associated with a neuropathy, is transient, and after it disappears many years may pass before an individual has any significant symptoms referable to HIV infection. Lymphadenopathy develops in 30–40% of infected individuals and this in itself is not a bad prognostic sign. As noted above, within ten years of infection with HIV, 67% of infected individuals have so many CD4 lymphocytes destroyed that they develop that ultimate complication of HIV infection: AIDS.

Prior to the development of AIDS, two HIV-related complications are common. The first is Kaposi's sarcoma, which is a tumour-like proliferation of the endothelial cells lining blood vessels[10]. The lesions often begin in the skin, although they are frequently first recognized in the mouth, particularly on the hard palate. The proliferation of these cells can occur within internal organs. Kaposi's sarcoma probably occurs when an as yet unidentified sexually transmitted cofactor stimulates endothelial cells to release cytokines which perpetuate the proliferation of these cells. Cytokines are like lymphokines, that are hormone-like substances released from cells, but in this case the chemicals referred to are released by cells lining blood vessels rather than lymphocytes. It is the limitation of Kaposi's sarcoma to homosexual men infected with HIV in the West that has led many to suggest that a sexually transmitted cofactor must be involved.

Kaposi's sarcoma can be cosmetically disfiguring but harmless, or can involve lymphatic tissue and produce gross swelling, particularly in the lower limbs and scrotum, and become a major clinical problem. Frequently, it displays a propensity for the nose and eyelids. Small lesions often respond to radiotherapy, but chemotherapy may be required if lesions are producing secondary phenomena of consequence.

The second complication is that some 10–20% of patients develop a presenile dementia prior to the development of AIDS. Initially, higher-centre functions are affected, such as concentration and judgment, and these defects can be measured using psychometric analysis. Antiretroviral therapy with AZT can minimize the symptoms of HIV-related dementia if introduced into the management of the condition soon after the onset of symptoms. This complication seems to be caused by certain strains of the virus that infect macrophages that transport the virus to the brain and then subsequently release cytokines that damage delicate nervous tissue. Macrophages are scavenger cells of the immune system and certain variations within the virus itself seem to make it easier for certain macrophages to swallow viral particles and transport them to the central nervous system.

Prior to the development of AIDS, as viral replication becomes more efficient, accelerated T cell destruction occurs, and patients may develop a period of weight loss characterized by profuse sweating at night and the development of a fungal infection in their mouth with *Candida albicans*.

CD4 lymphocytes are usually present in the blood in numbers greater than 700 µl. When the number falls to 200 µl or less, patients are at risk of developing opportunistic infections, which lead to the definition of AIDS in an individual case. Pneumonia produced by *Pneumocystis carinii* is the most common AIDS-defining illness. Toxoplasmosis, *Cryptococcus neoformans* infection (usually of the central nervous system), atypical and typical tuberculosis (the latter increasingly drug-resistant) and problems associated with cytomegalovirus are some of the most common serious infections noted. Once AIDS develops, patients usually survive no longer than two years even when the most sophisticated treatment is available.

Antiretroviral therapy

While numerous drugs to help manage HIV infection are in various stages of development, only one family of

drugs have so far been shown to be successful and relatively safe after extensive clinical trials. AZT was the first drug developed that could inhibit the replication of HIV effectively. As with all of the drugs that are currently available, AZT works by interfering with reverse transcriptase and by interfering with the successful transcription of viral DNA into viral RNA. Recent studies reveal that none of the currently available antiretroviral agents are effective long-term, as in most patients, viral mutations lead to drug resistance within 12–18 months. This appears to be true whether monotherapy or combination therapy is used. Strategies being studied now involve the rotation of various retroviral agents on a regular basis and the introduction of new drugs which attack viral replication at different stages of the virus's life cycle.

Vaccines

To date no vaccine has been developed that can prevent infection with HIV in humans or chimpanzees, the only animal besides humans that can be infected with HIV-1 or HIV-2. Although clinical trials in humans of preventative vaccines have started, no clinical data related to their efficacy is as yet available. Somewhat dishearteningly, evidence suggests that the vaccines used to prevent the infection of monkeys with a simian immunodeficiency virus (SIV) infection have been ineffective. Certain monkeys infected with that virus develop AIDS. Epidemiological investigations in the last twelve months have noted the emergence of more and more new strains of HIV in various parts of the world. There are now more than nine major variations on a theme which will make it extremely difficult to produce a vaccine that will be equally effective in different parts of the world. The difficulty in producing a cheap effective vaccine highlights the need to reemphasize preventative strategies. Because preventing infection is proving to be so difficult, many scientists have turned their attention to preventing disease. HIV infection does not produce significant clinical consequences in the first few years of infection. If the initially adequate immune response could be so strengthened that HIV replication is permanently minimized, then HIV infection may be no more serious than infection with the virus responsible for chicken pox. That virus is never eliminated from the body, but after an initial flourish rarely causes further problems. Therapeutic vaccines have been developed and are being tested in an attempt to introduce to a competent immune system viral envelope and core variations that the immune system may encounter at a later date during the course of HIV infection. While it is too early to judge the success of this strategy, on theoretical grounds it seems to be likely to produce more immediate benefits than any other therapeutic strategy currently being developed.

Summary

Two distinct members of the retrovirus family, HIV-1 and HIV-2, can selectively destroy CD4 lymphocytes in humans, and thus over a period of ten years can induce a degree of immunodeficiency that allows opportunistic infections and the development of malignancy to kill infected individuals. Envelope proteins developed by the virus provide numerous mechanisms for the destruction of immunologically competent cells. 80% of infections occur during vaginal intercourse, with blood-to-blood transmission being responsible for most remaining infections. At this time, anal intercourse is responsible for less than 10% of infections worldwide. No effective vaccine to prevent infection is likely to be developed within the next ten years. Prevention must remain the major goal of all public health officials, or the World Health Organization's prediction that 30–40 million people will be infected by the year 2000 may turn out to be a significant underestimate. Antiretroviral drugs and therapeutic vaccines offer the best hope in the immediate future for prolonging quality life in people infected with HIV.

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