CASE 13 AIDS in Mother and Child

Infection can suppress adaptive immunity.

Certain infectious microorganisms can suppress or subvert the immune system. For example, in lepromatous leprosy, *Mycobacterium leprae* induces T cells to produce lymphokines that stimulate a humoral response but suppress the development of a successful inflammatory response to contain the leprosy bacillus. The leprosy bacillus multiplies and there is a persistent depression of cell-mediated immune responses to a wide range of antigens (see Case 34). Another example of immunosuppression is provided by bacterial super-antigens, such as toxic shock syndrome toxin-1. Super-antigens bind and stimulate large numbers of T cells by binding to certain V_β chains of the T-cell receptor, inducing massive production of cytokines by the responding T cells (see Case 7). This, in turn, causes a temporary suppression of adaptive immunity.

Topics bearing on this case:
Failure of cell-mediated immunity
Infection with the human immuno-deficiency virus (HIV)
Control of HIV infection
Drug therapy for HIV infection
ELISA test

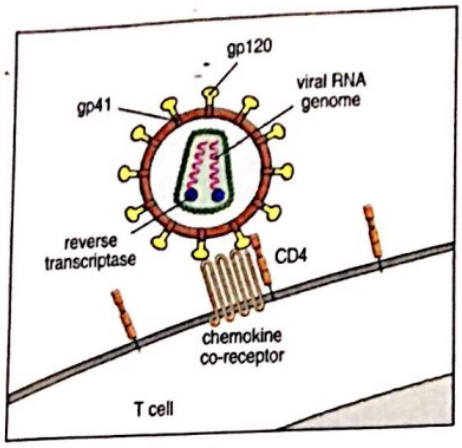


Fig 13.1 HIV binds to CD4 T cells through its coat glycoprotein gp120. The gp120 molecule on the surface of the virus binds CD4 on T cells and macrophages; the viral protein gp41 then mediates fusion of the enveloped virus with the target cell, allowing the viral genome to enter the cell.

At the beginning of this century, when tuberculosis was the leading cause of death and fully half the population was tuberculin-positive, it was well known that an intercurrent measles infection would cause a well-contained tuberculosis infection to run rampant and result in death. The mechanism responsible is now known to be the suppression of IL-2 synthesis after binding of measles virus to CD46 on macrophages.

Some of the microorganisms that suppress immunity act by infecting lymphocytes. Infectious mononucleosis or glandular fever is caused by a virus (Epstein–Barr virus) that infects B lymphocytes. The infection activates cytotoxic CD8 T cells, which destroy the B cells in which the Epstein–Barr virus is replicating. In the third week of infection, at the height of activation of CD8 T cells, all adaptive immunity is suppressed. The lymphokines responsible for the immunosuppression are not well defined but probably include interleukin-10 and TGF-β (see Case 14).

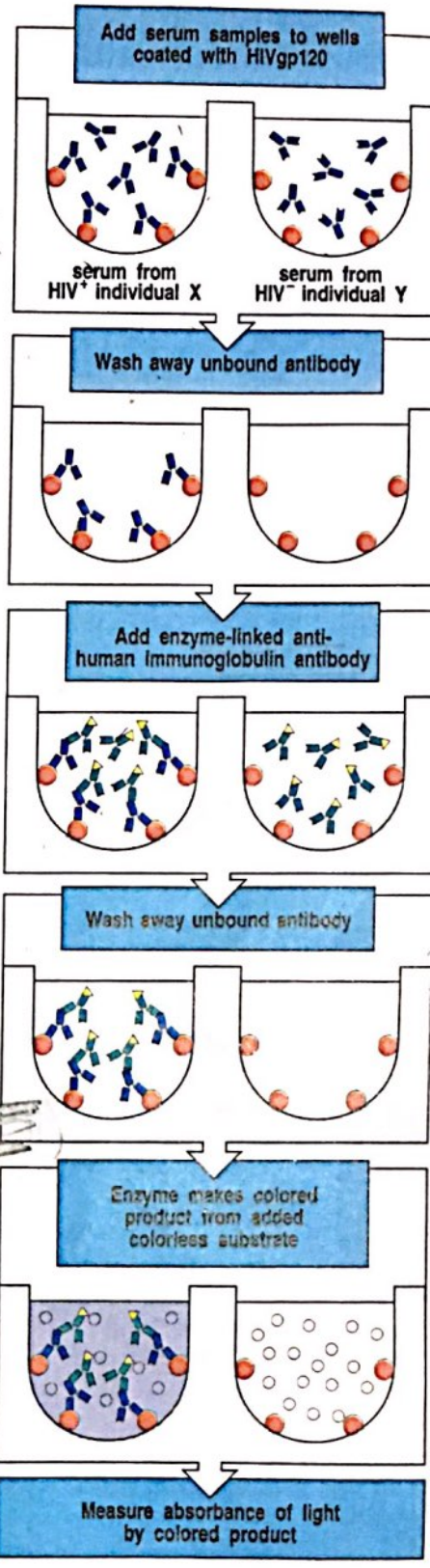
The human immunodeficiency virus (HIV) presents a chilling example of the consequences of infection and destruction of immune cells by a microorganism. The T-cell surface CD4 molecule acts as the receptor for HIV (Fig. 13.1). CD4 is also expressed on the surface of cells of the macrophage lineage and they too can be infected by this virus. The chemokine receptors CCR5 and CXCR4 act as obligatory co-receptors for HIV. As we shall see, the primary infection with HIV may go unnoticed, and the virus may replicate in the host for many years before symptoms of immunodeficiency can be seen. During this period of clinical latency, the level of virus in the blood and the number of circulating CD4 cells remains fairly steady but in fact both virus particles and CD4 cells are being rapidly destroyed and replenished, as rounds of virus replication take place in newly infected cells. When the rate at which CD4 cells are being destroyed exceeds the capacity of the host to replenish them, their number decreases to a point where cell-mediated immunity falters. As we have seen in other cases, such as severe combined immunodeficiency (see Case 5), the failure of cell-mediated immunity renders the host susceptible to fatal opportunistic infections.

The Pinkerton family: a tragedy from contaminated blood.

Benjamin Pinkerton was a captain in the United States Navy, stationed in Japan. He married a Japanese woman before leaving Japan for his new assignment in Honolulu, Hawaii. His wife, Chieko, gave birth to a healthy daughter in 1987. Two years later they had a son, Franklin, who weighed 8 pounds at birth and appeared to be very healthy. At age 3, 4, and 5 months, Franklin received routine immunization with tetanus and diphtheria toxoids and pertussis bacteria (DPT) as well as oral polio vaccine. He had no reactions to these inoculations and seemed to be thriving. At 6 months of age he became sick and lost weight. He developed severe, persistent diarrhea with fever. He was noted to have white spots (thrush) in his mouth; he had infections of the middle ear (otitis media) twice in rapid succession. Franklin was seen several times at the Naval Hospital by pediatricians who treated him with antibiotics but he did not seem to get better. At 7 months of age Franklin developed mild difficulty in breathing.

Six-month-old infant with thrush, diarrhea, and otitis media.

Fig. 13.2 Use of the enzyme-linked immunosorbent assay (ELISA) to detect the presence of antibodies to the HIV coat protein gp120. Purified recombinant gp120 is coated onto the surface of plastic wells to which the protein binds nonspecifically; residual sticky sites on the plastic are blocked by adding irrelevant proteins (not shown). Serum samples from the individuals being tested are then added to the wells under conditions where nonspecific binding is prevented, so that only binding to gp120 causes antibodies to be retained on the surface. Unbound antibody is removed from all wells by washing, and anti-human immunoglobulin that has been chemically linked to an enzyme is added, again under conditions that favour specific binding alone. After further washing, the colourless substrate of the enzyme is added, and colored material is deposited in the wells in which the enzyme-linked anti-human immunoglobulin is found. This assay allows arrays of wells known as microtiter plates to be read in fiberoptic multichannel spectrometers, greatly speeding the assay.



On physical examination at this time his temperature was recorded at 38°C (normal 37°C). Thrush was evident as white plaques (*Candida* spp.) on the buccal mucosa (the inside of his mouth) and anterior surface of his tongue (see Fig. 5.4). A diaper rash was also present that seemed to be due to *Candida* infection. He had fine inspiratory rales (crackles) in both lungs. He was admitted to the hospital, where his white blood cell count was found to be normal at 6500 μl^{-1} with a normal differential count of 62% neutrophils, 5% monocytes, 30% lymphocytes, 2% eosinophils, and 1% basophils. His serum immunoglobulin G measured 997 mg dl^{-1} (normal), IgM 73 mg dl^{-1} (normal), and IgA 187 mg dl^{-1} (normal). An examination of his lymphocytes revealed 1825 CD8 T cells μl^{-1} (normal) but only 85 CD4 T cells μl^{-1} (very depressed). He showed no delayed-type hypersensitivity (DTH) response to intradermal *Candida* antigen or to PPD (purified protein derivative of tuberculosis). His serum contained antibodies to HIV by ELISA testing (Fig. 13.2) and Western blot (Fig. 13.3). After this had been discovered, his mother, father, and sister were also tested by the same methods. His mother and father tested positive for antibodies to HIV. His sister tested negative.

Although Captain Pinkerton was in good health, his wife, Chieko, had felt run down, and complained of low-grade fevers and swollen lymph nodes in her neck. She attributed all these symptoms to the stress of Franklin's illness. However, it turned out that a year before Franklin's birth, Chieko had been pregnant. Near the end of her pregnancy the fetus died and had to be removed by Caesarian section. She recovered from the Caesarian section surgery and felt perfectly well, but because of blood loss during the surgery, she had been given 2 units of blood.

In the hospital, bronchial washings were obtained from Franklin. A stain for *Pneumocystis carinii* was positive and he was treated with the drugs trimethoprim and sulfa, which cleared the infection. Nonetheless, Franklin did not reach his developmental milestones on schedule. He was unable to crawl at 10 months. He became unsteady when sitting. He lost 1 kg of body weight. Despite the successful eradication of his *Pneumocystis carinii* infection, Franklin's breathing became more rapid and his lungs appeared worse on radiographs. Culture of tissue from an open lung biopsy grew cytomegalovirus, respiratory syncytial virus, and *Pseudomonas aeruginosa*. A duodenal biopsy also contained cytomegalovirus. He developed a severe cough and was spitting up blood (hemoptysis). One week later he died of respiratory failure.

Chieko, in the meanwhile, was started on AZT (zidovudine) therapy to combat her HIV infection but she developed *Pneumocystis carinii* pneumonia when her CD4 T-cell number fell below 200 μl^{-1}. Despite successful treatment with trimethoprim and sulfa, the infection recurred. She died 5 months after Franklin from respiratory failure. Captain Pinkerton has remained asymptomatic despite the persistence of HIV antibodies in his serum.

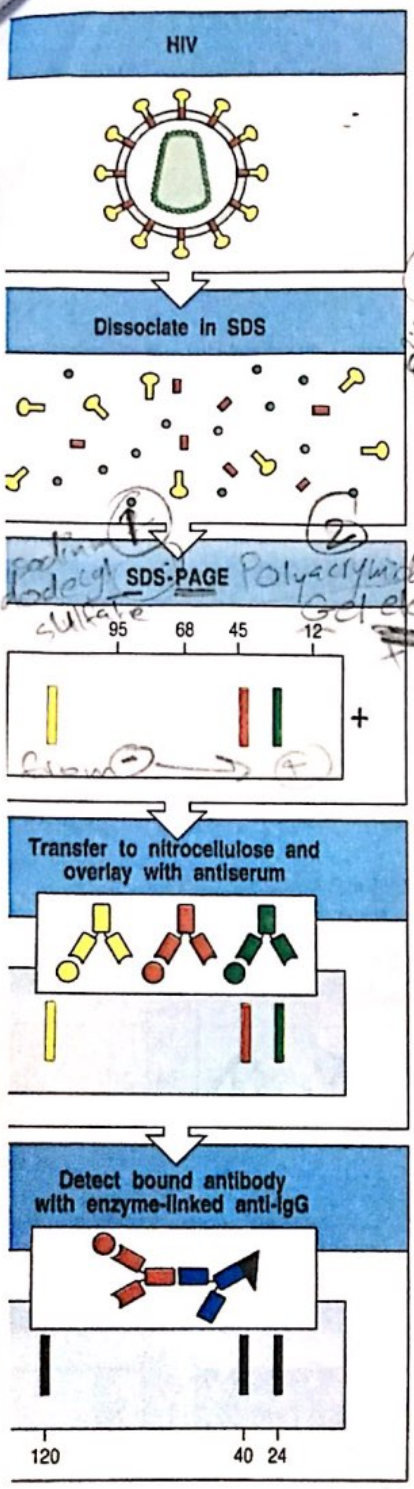


Fig. 13.3 Western blotting is used to identify antibodies to the human immunodeficiency virus (HIV) in serum from infected individuals. The virus is dissociated into its constituent proteins by treatment with the detergent SDS, and its proteins are separated by SDS-PAGE. The separated proteins are transferred to a nitrocellulose sheet and reacted with the test serum. Anti-HIV antibodies in the serum bind to the various HIV proteins and are detected by using enzyme-linked anti-human immunoglobulin, which deposits colored material from a colorless substrate. This general methodology will detect any combination of antibody and antigen and is used widely, although the denaturing effect of SDS means that the technique works most reliably with antibodies that recognize the antigen when it is denatured.

Acquired Immune Deficiency Syndrome (AIDS)

AIDS is caused by the human immune deficiency virus (HIV) of which there are two known types, HIV-1 and HIV-2. HIV-2 was largely confined to West Africa but now seems to be spreading into Southeast Asia. HIV infections in North and South America and in Europe are exclusively from HIV-1. HIV can be transmitted by homosexual and heterosexual intercourse, by infusion of contaminated blood or blood products, or by contaminated needles, which are the major source of infection among drug addicts. The infection can also be passed from mother to child during pregnancy, during delivery or, more uncommonly, by breastfeeding. Somewhere between 25% and 35% of infants born to HIV-positive mothers are infected. The rate of infection of infants has been decreased fourfold by giving HIV-positive pregnant women the anti-viral agent AZT (zidovudine).

Contact with the virus does not necessarily result in infection. The standard indicator of infection is the presence of antibodies to the virus coat protein gp120. The initial infection, as in Chieko's case, may pass unnoticed and without symptoms. More often a mild viral illness within 6 weeks of infection is sustained, with fever, swollen lymph nodes, and a rash. It subsides at about the time that seroconversion occurs, and although virus and antibody persist, the patient feels well. A period of clinical latency lasting years, and perhaps even decades, may ensue during which the infected person feels perfectly well. Then he/she begins to experience low-grade fever and night sweats, excessive fatigue, and perhaps oral candidiasis (thrush) in the mouth. Lymph nodes in the neck or axillae or groin may swell. Weight loss may become very marked. These are the prodromal symptoms of impending AIDS. (A prodrome is a concatenation of signs and symptoms that predict the onset of a syndrome.) The number of CD4 T cells in the blood may have been normal up to this time but, with the onset of the prodrome, the CD4 T-cell count begins to fall (Fig. 13.4). When the number of CD4 T cells decreases to the range of 200–400 cells μl^{-1}, the final phase of the illness, which is called AIDS, starts. At this time serious, eventually fatal, opportunisitic infections as well as certain unusual malignancies occur (Fig. 13.5).

At any time after the infection, HIV may infect megakaryocytes, which have some surface CD4. Because megakaryocytes are the bone marrow progenitors of blood platelets, extensive infection of megakaryocytes causes the platelet count to fall (thrombocytopenia) and bleeding to occur. HIV may also infect the glial cells of the brain. Glial cells are of monocyte–macrophage lineage and have some CD4 on their surface. The infection of glial cells may cause dementia and other neurological symptoms, such as the motor problems and developmental retardation seen in Franklin.

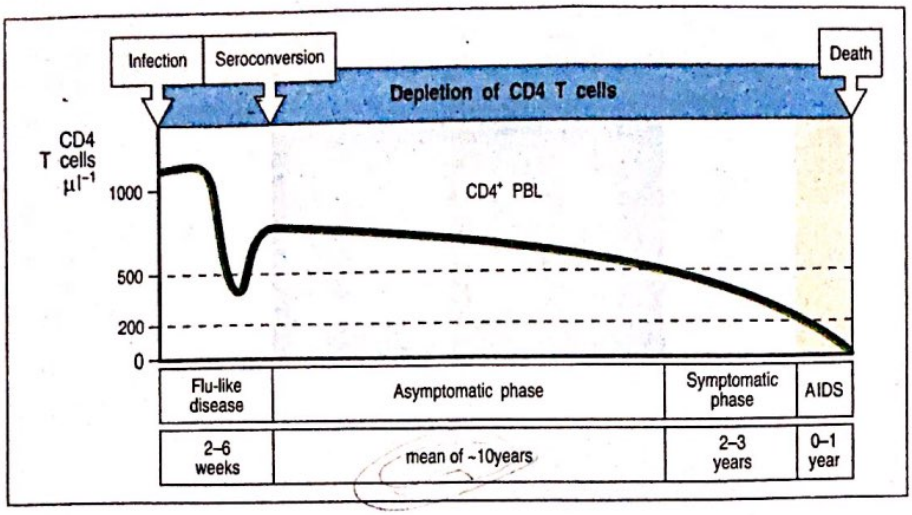


Fig. 13.4 The typical course of infection with HIV. The first few weeks are typified by an acute influenza-like viral illness, sometimes called seroconversion disease, with high titers of virus in the blood. An adaptive immune response follows, which controls the acute illness and largely restores CD4 T cell levels but does not eradicate the virus. Opportunistic infections and other symptoms become more frequent as the CD4 T-cell count falls, starting at around 500 cells μl^{-1}. The disease then enters the symptomatic phase.

Discussion and questions.

1 *When Franklin was seen by the pediatricians at the Naval Hospital, their first impression was that he had severe combined immunodeficiency (SCID) because of the cough, thrush, and persistent diarrhea. What laboratory findings directed their attention to the diagnosis of AIDS?*

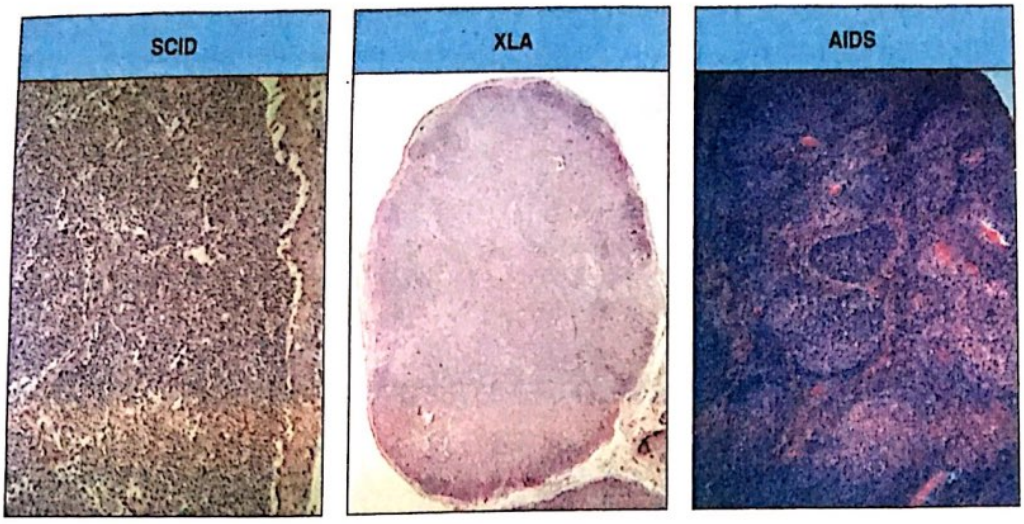
His serum immunoglobulin levels were normal. In SCID they would be very low. His T cells showed a specific deficit of $CD4^+$ T cells. In SCID the total number of T cells would be decreased.

2 *If a lymph-node biopsy had been obtained from Franklin, in what way would it differ from the histopathology of a lymph node if he had SCID or X-linked agammaglobulinemia (XLA)?*

The lymph node would be enlarged and exhibit marked, if not exuberant, follicular hyperplasia. In SCID and XLA the lymph nodes are very small. In SCID, the node would contain no or very, very few lymphoid cells. In XLA, the lymph node would have no follicles, no germinal centers, and no B cells or plasma cells. T cells would be present but not in an organized array (Fig. 13.6).

Infections	
Parasites	*Toxoplasma* spp. *Cryptosporidium* spp. *Leishmania* spp. *Microsporidium* spp.
Bacteria	*Mycobacterium tuberculosis* *Mycobacterium avium intracellulare* *Salmonella* spp.
Fungi	*Pneumocystis carinii* *Cryptococcus neoformans* *Candida* spp. *Histoplasma capsulatum* *Coccidioides immitis*
Viruses	Herpes simplex Cytomegalovirus Varicella zoster
Malignancies	
Kaposi's sarcoma (invasive) Non-Hodgkin's lymphoma, including EBV-positive Burkitt's lymphoma Primary lymphoma of the brain	

Fig. 13.5 A variety of opportunistic pathogens and cancers can kill AIDS patients. Infections are the major cause of death in AIDS, with respiratory infection with *Pneumocystis carinii* being the most prominent. Most of these pathogens require effective macrophage activation by CD4 T cells or effective cytotoxic T cells for host defense. Opportunistic pathogens are present in the normal environment but cause severe disease primarily in immunocompromised hosts, such as AIDS patients and cancer patients. AIDS patients are also susceptible to several rare cancers, such as Kaposi's sarcoma and lymphomas, suggesting that immune surveillance by T cells may normally prevent such tumors. EBV, Epstein–Barr virus.



3 *The course of the HIV infection was very different in Franklin and in his mother and illustrates the differences between pediatric AIDS and adult AIDS. What is the major difference and how do you account for it?*

In infants, the HIV infection typically runs a more rapid course. Franklin died before he reached 1 year of age. His mother was infected but without symptoms for 4 years. The difference in the course of AIDS in infants and in adults is probably due to the fact that the infection occurs in an immunologically immature and naive subject when it affects infants, whereas an infected adult has a functionally mature immune system and decades of acquired adaptive immunity. This has consequences for both the response to HIV itself, and the susceptibility to other infections. We have seen that newborn T cells are not fully 'turned on.' For example, in the hyper IgM case (see Case 3), we saw that the CD40 ligand is not readily expressed on activation of the T cells of newborns. Their T cells do not synthesize interferon-γ in normal amounts. Their cytotoxic T lymphocytes are not readily activated. This functional immaturity is probably why young infants have difficulty confining and walling off infections, particularly those that require adaptive immunity mediated by T cells. Tuberculosis offers a clear example of this. It is a fast-spreading, highly lethal infection in young infants, whereas in older children and adults who are immunologically normal, this infection is usually confined to the lung, or more rarely to other organs. HIV infection in infants occurs before they have had an opportunity to develop any adaptive immunity to common infections, and this means they are prone to certain infections not seen in adult AIDS. Thus an adult will already have antibodies to the common pyogenic bacteria and will not be particularly susceptible to pyogenic infections, whereas these are frequently observed in affected infants. Adults will also have been exposed to common viruses. Epstein–Barr Virus (EBV), for example, is normally encountered early in life and contained as a latent infection. Virtually all adults have been infected with EBV by the end of the second or third decade of life, and primary EBV infection is therefore not a threat to HIV-infected adults. For an HIV-infected infant, however, a first encounter with this virus causes bizarre manifestations such as parotitis (inflammation of the parotid gland, like mumps) and a form of pneumonia characterized by pulmonary lymphoid hyperplasia (Franklin did not have these, or any other evidence of a primary EBV infection).

4 *What are the mechanisms of resistance to the progression of HIV infection?*

The answer to this question is not known precisely. The immune response to the virus is illustrated in Fig. 13.7. Antibody to HIV seems to play a minor role in resisting the progress of infection. HIV-specific cytotoxic CD8 cells arise as virus levels decline from the peak associated with primary infection, and seem to have a more important role in containing the infection. Rare individuals with mutations in the co-receptors for HIV (CCR5 and CXCR4) are resistant to HIV infection.

5 *A few individuals, mostly hemophiliacs, are known to have been infected with HIV as long as 20 years ago and yet they remain asymptomatic. What factors may contribute to long-term survival with this infection?*

The virus burden in these individuals is very low and, in some cases, the only detectable viruses carry mutations in genes such as *nef* or *tat*, which are vital to HIV replication in the infected host. However, viruses able to replicate in culture can be isolated from most of these so-called 'long-term non-progressors.' These patients seem to contain replication-competent virus, most probably by continuing to maintain a successful cytotoxic CD8 T-cell response.

6 *What is the mode of action of AZT or zidovudine?*

AZT (3′-azido,2′,3′-dideoxythymidine) is a nucleotide analogue that is phosphorylated inside the cell, and used as a substrate by the reverse transcriptase of HIV (Fig. 13.8). HIV reverse transcriptase synthesizes a DNA complement of the viral RNA, at the start of a new round of virus replication in a newly infected cell. The incorporation of AZT blocks further extension of this DNA strand and thereby stops replication of the virus. Two other nucleotide analogues, ddI (dideoxyinosine) and ddC (dideoxycytosine) inhibit HIV replication by a similar mechanism and are also used to treat HIV infection. Unfortunately, mutation allows the virus to acquire resistance to all these drugs. Replication of HIV (and other known retroviruses) is error-prone, and the virus mutates as it replicates in the infected host. Resistance to AZT requires multiple mutations but can arise in only a few months. Combining HIV protease inhibitors with zidovudine or other nucleotide analogues has dramatically improved the survival of HIV-infected patients.

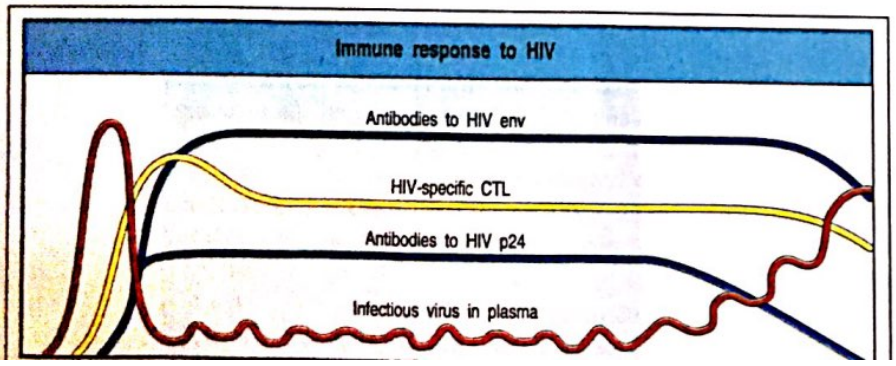


Fig. 13.7 The immune response to HIV. Infectious virus is present at relatively low levels in the peripheral blood of infected individuals during a prolonged asymptomatic phase but is replicated persistently in lymphoid tissues. During this period, CD4 T-cell counts gradually decline, although antibodies and CD8 cytotoxic T cells directed against the virus remain at high levels. Two different antibody responses are shown in the figure, one to the envelope protein of HIV, env, and one to the core protein p24. Eventually, the levels of antibody and HIV-

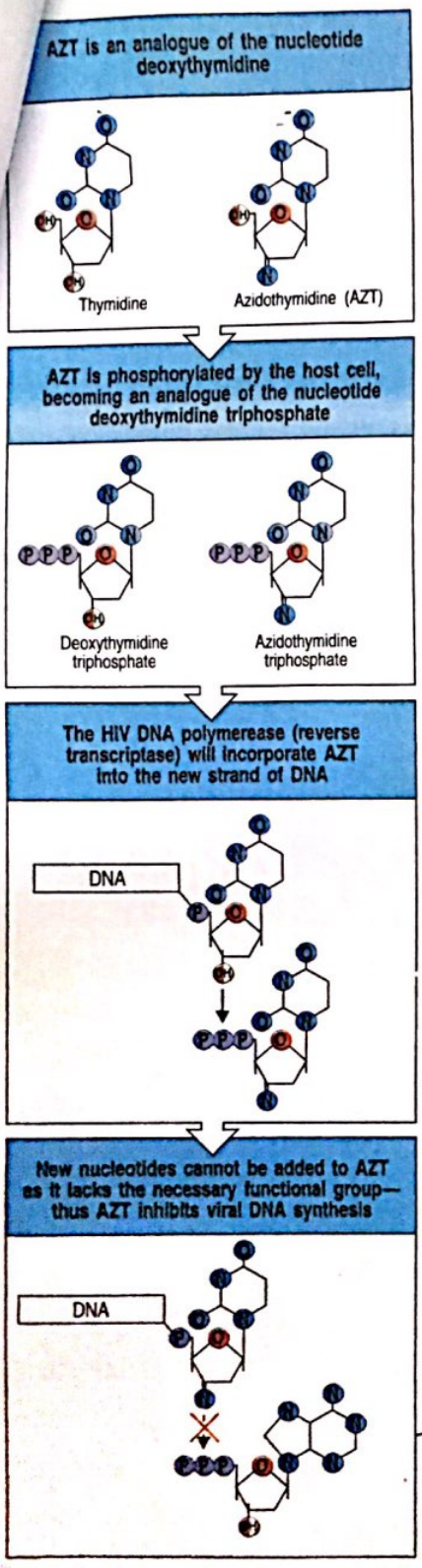


Fig. 13.8 The mechanism of action of the AIDS drug AZT.

7 *What is the mechanism of CD4 T-cell depletion in HIV infection?*

The precise answer to this question is also unknown. It is clear that the cells producing the virus are killed, either by cytotoxic T lymphocytes or by direct cytotoxic effects of the virus. It is also possible that the death of noninfected 'bystander' cells contributes to CD4 T-cell depletion. HIV is known to have a cytotoxic effect on CD4 T cells in culture. The viral capsular gp120 binds and cross-links the CD4 molecule, which depresses T-cell function and may induce apoptosis, even when the CD4 cells are not themselves infected by HIV. If, as seems likely, the early killing of HIV-infected CD4 cells by cytotoxic T cells serves to contain the virus and prevent greater CD4 cell depletion in the next round of HIV infection and replication, a declining ability to mount cytotoxic responses, especially to new viral variants, could be very important. Patients infected with HIV show impairment of T-cell function, especially memory cell responses, even during the asymptomatic phase. They are hypergammaglobulinemic, and humoral responses seem favored at the expense of cell-mediated immunity. This immunoregulatory bias impedes inflammatory responses, and possibly also cytotoxic responses to HIV-infected cells.

8 *What is the most important known determinant of the progression of HIV infection?*

The CD4 T-cell count is, statistically speaking, the best indicator of the time-course of progression to AIDS. Other factors such as lifestyle and the incidence of intercurrent infections do not seem statistically significant.

9 *Which cytokine, released in HIV infection, causes weight loss?*

Tumor necrosis factor-alpha (TNF-α). It causes loss of appetite (anorexia) and increased expenditure of body heat (thermogenesis).

10 *What steps have been taken since the early 1980s to prevent a tragedy such as occurred in the Pinkerton family? What pitfalls are encountered in screening blood and blood products?*

All blood banks screen blood donations for antibody to HIV gp120 by enzyme-linked immunosorbent assay (ELISA) and confirm positive results with Western blotting. The major pitfall in relying on this approach is its inability to detect an HIV-infected blood donor in the period between acquiring HIV and the formation of antibody to HIV. Such individuals can be detected only by testing blood for HIV by polymerase chain reaction (PCR).