

## MINIREVIEWS

# Current Concepts in Human Immunodeficiency Virus Infection and AIDS

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The global epidemic of AIDS has become one of the most pressing public health emergencies of this century. Initial reports of AIDS date back to 1981. However, current data suggest that AIDS has existed for at least several decades. While both forms of the human immunodeficiency virus (HIV), type 1 and type 2, are retroviruses capable of causing fatal AIDS, infection with the latter generally results in a longer incubation period and a more indolent course of disease. Maternal-fetal transmission of HIV-2 is limited, and infection with HIV-2 seems to provide natural protection, estimated at approximately 70%, against infection with HIV-1 in certain high-risk groups (50). HIV-2, initially endemic to west Africa, also is spreading worldwide.

Retroviruses encode their genetic information as RNA which, upon infection of a host cell, is reverse transcribed into double-stranded DNA. The latter is integrated into the DNA of the host cell chromosomes. Other examples of human retroviruses include the human T-cell lymphotropic viruses types I and II (HTLV-I and -II). HTLV-I is the etiologic agent for adult T-cell leukemia and tropical spastic paraparesis, also known as HTLV-I-associated myelopathy. Hairy cell leukemia, a B-cell malignancy, has been linked to HTLV-II (14). HIV-1 infections produce a variety of manifestations such as immunodeficiency with accompanying opportunistic infections, malignancies, severe cachexia, and encephalopathy including dementia (49).

Progress has been made in the treatment of HIV infections. Nucleoside analogues that inhibited viral reverse transcriptase (RT) were the first class of antiretroviral drugs but the emergence of resistant strains limited their effectiveness. The recent introduction of protease inhibitors to our current armamentarium, combined with multidrug therapy, has yielded encouraging results (10, 13, 20). Nevertheless longitudinal outcome data, including assessment of the emergence of drug-resistant mutants, are needed to determine the long-term potential of current therapy.

### EPIDEMIOLOGY

The latest statistics provided by the Joint United Nations Programme on HIV/AIDS (UNAIDS) record 33.4 million people living with HIV/AIDS worldwide (1a). This can be stratified into 32.2 million adults, of whom 13.8 million are women, and 1.2 million children under the age of 15. Since the

beginning of the epidemic, there has been 13.9 million deaths worldwide due to AIDS, including 3.2 million children. Within the 50 United States a cumulative total of 687,397 cases of AIDS were reported to the Centers for Disease Control and Prevention (CDC) through the end of 1998 (12).

The first reports of AIDS in the United States occurred in June 1981. Initially, AIDS prevalence seemed to be restricted to homosexual men and patients with hemophilia. However, HIV infections are primarily a sexually transmitted disease (STD) that has no preference for any sexual orientation. HIV infections may have existed for at least several decades in central Africa, where the male to female prevalence ratios are about equal. HIV-2 demonstrates a closer genetic relationship and geographic distribution to the simian immunodeficiency virus (SIV), long endemic to central Africa, than to HIV-1 (38). Sequencing studies of early HIV-2 isolates showed a 75% nucleic acid homology with SIV but only a 40 to 50% homology with HIV-1. Thus, it was hypothesized that HIV-2 may be the prototype virus that was originally transmitted from monkeys to man.

Transmission of HIV can occur through contact with infected body fluids. It is currently assumed that the relatively cellular body fluids such as blood, semen, vaginal secretions, and breast milk are more effective in transmitting the virus than fluids deficient in cells such as saliva, urine, and tears. Transmission may occur across mucous membranes or broken skin during sexual intercourse (both heterosexual and homosexual), but it also may occur via intravenous exposure such as through sharing infected needles with intravenous drug use, occupational exposure in the health care environment, or treatment with infected blood products. Now that donated blood is routinely screened for HIV in developed and many developing countries, the potential for acquisition of HIV via this route has been reduced significantly. In the United States recent estimates of the risk of transmission of HIV by blood transfusion ranged from 1 in 450,000 to 1 in 660,000. The significance of exposure risk to HIV-1 is summarized in Table 1. Vertical transmission from an infected mother to her child is a special problem. Infection can occur in utero or intrapartum. Postpartum infection can result from the ingestion of breast milk by a nursing infant from an infected mother (53). It has been estimated by the World Health Organization that up to one-third of all cases of transmission of HIV-1 from an infected mother to her child may occur via breast milk. However, for certain populations where the infant mortality rate is high, the risk of death associated with the lack of breast feeding may be greater than the risk of HIV infection acquired through breast milk (7).

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TABLE 1. HIV infection cases by age group, exposure category, and sex<sup>a</sup>

Exposure category	No. (% of total) by sex and age group		
	Male	Female	Pediatric
Male homosexual exposure	317,862 (57)		
Injection drug use	122,933 (22)	45,075 (43)	
Male homosexual exposure and injection drug use	42,093 (8)		
Hemophilia or coagulation disorder	4,559 (1)	222 (0)	234 (3)
Heterosexual exposure	21,888 (4)	40,744 (39)	
Blood transfusion	4,752 (1)	3,559 (3)	375 (5)
Unknown	38,999 (7)	14,428 (14)	159 (2)
Maternal transmission			7,512 (91)

<sup>a</sup> Data are from reference 12a and are cumulative totals to June 1998. Pediatric group comprises individuals <13 years of age. Individuals in male and female groups are ≥13 years of age.

### BIOLOGY OF HIV INFECTION

HIV is a human retrovirus consisting of a noncomplementary pair of single coding or positive (+) strands of RNA enclosed within an inner, nucleocapsid, protein core surrounded by a lipid bilayer envelope. Various host cell proteins are incorporated into the lipid bilayer of the viral envelope. An envelope glycoprotein with a molecular size of 41 kDa (gp41) is anchored within the lipid bilayer to which gp120 is noncovalently attached (28). The crystal structure of gp120 in complex with the CD4<sup>+</sup> receptor and a neutralizing human antibody was recently determined to a resolution of 2.5 Å (33). Figure 1 shows a diagram of the structure of HIV. The genetic map of HIV is depicted in Figure 2.

The binding of HIV to target cells was previously thought to only involve interaction of gp120 of the virus envelope with the CD4 molecule on the cell membranes of T-helper lymphocytes, monocytes and macrophages, and microglia in the brain (49). However, recently it was shown that efficient viral entry requires a coreceptor that is also the natural receptor for the β chemokines, a family of chemotactic cytokines (35). This will be discussed further below. After binding, the viral envelope fuses with the membrane of the target cell and the contents of the virus enter the cytoplasm. Molecules of the virus-derived enzyme, RNA-dependent DNA polymerase or RT, are released into the target cell together with the viral RNA. RT then directs the synthesis of a complementary strand of DNA on the RNA template from the virus. Thereafter the RT directs the synthesis of a second strand of DNA complementary to the initial DNA strand. RT is highly error prone through substitution of incorrect bases, accounting for the high mutation rate of HIV. Viral nucleic acids including RNA and DNA form a high-molecular-weight preintegration complex with HIV proteins (e.g., RT, integrase, and matrix protein) which is transported to the nucleus. The double-stranded DNA copy of the viral RNA is then randomly integrated into the host's DNA. The proviral form of HIV integrated into the host chromosome is generally quiescent and replicates coordinately with the host cell DNA. When the HIV-infected cell undergoes activation or stimulation, the provirus is transactivated, resulting in the production and release of infectious virions. The life cycle of HIV is shown in Fig. 3.

HIV can be broadly divided into two phenotypes: one induces syncytium formation among infected cells and the other does not (24). The syncytium-inducing (SI) strain readily infects T lymphocytes, replicates at a high rate, and is associated with a relatively more rapid course of the disease. The non-syncytium-inducing (NSI) phenotype shows tropism for mac-

rophages, replicates slowly, and is not associated with rapid progression. The V1 and V2 loops of gp 120 regulate virus entry into the host, whereas the V3 loop is the major determinant of tropism for the SI and NSI phenotypes (31, 47, 52). Syncytium formation is enhanced in the CD45RO<sup>+</sup> memory subset of CD4<sup>+</sup> lymphocytes (29). Syncytia have a shorter half-life and tend to die more quickly than individual infected cells in vitro; however, this has not been demonstrated in vivo. SI strains are more frequently isolated from patients with full-blown AIDS, whereas NSI strains tend to predominant in recently infected patients. These observations suggest that the NSI phenotype is more likely to produce primary infections. This may be due to the fact that macrophages are highly susceptible to the NSI phenotype and can serve as a reservoir for HIV. In some patients, however, the asymptomatic phase may be shortened concomitant with a shift in the viral phenotype from NSI to SI. This shift in phenotype may be distinguished on the basis of CD4 counts and quantitative measurement of HIV RNA. The envelope protein of the SI strain exhibits a high affinity for the CD4<sup>+</sup> receptor, subsequently initiating syncytium formation and target cell destruction.

**Pathogenesis.** Although the course of HIV-1 infection can vary widely from patient to patient, a generalized clinical course is presented in Fig. 4. Fifty to 70% of patients develop an acute mononucleosis or flu-like syndrome within 3 to 6 weeks of primary infection, characterized by high levels of viremia and a significant drop in the absolute number of CD4<sup>+</sup> cells in the peripheral blood. The acute viremia is followed by activation of CD8<sup>+</sup> cells, which may be a response to control virus replication by cytotoxicity against infected target cells or through production of a soluble mediator that can suppress viral replication. During this stage, HIV is widely disseminated to the lymph nodes and induces a rapid turnover of infected lymphocytes (32, 49). It has been estimated from a mathematical model that the rate of virus production during active infection with HIV-1 is  $10.3 \times 10^9$  virions per day, and productively infected cells have a life-span of 2.2 days (22, 44). The extraordinary high production of virions and the disproportionate daily loss of CD4<sup>+</sup> cells led to what has become known as the "sink model" for CD4<sup>+</sup> cell loss (2).

Infection with HIV-1 induces a number of host responses including polyclonal activation of B lymphocytes, production of neutralizing antibodies, binding of immune complexes to follicular dendritic cells, synthesis and secretion of various cytokines, activation of Th-1 cells, and stimulation of cytotoxic responses including T-cell-, NK cell-, and antibody-dependent cell-mediated activities. While these responses can significantly reduce the viral load in the peripheral blood, generally they are unable to completely clear the infection. This early phase of HIV infection is followed by an intermediate stage or clinical latency that can last for several years (up to 12 years in some patients) and is characterized by gradual deterioration of immune responses and depletion of peripheral CD4<sup>+</sup> cells. Although the level of infectious virus in the peripheral blood is usually relatively low during this period, viral titers do not reach and stay at a nadir as previously thought. Rather, they rise steadily as the absolute number of peripheral CD4<sup>+</sup> T cells decreases. Viral replication continues at a very high rate in the lymph nodes, resulting in partial disruption of the germinal centers and the follicular dendritic cells. This is followed by massive production of cytokines such as tumor necrosis factor (TNF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-6 (IL-6) and a shift in the predominance of Th-2 over Th-1 responses. A decline of CD4<sup>+</sup> T cells below an absolute count of 50/μl is associated with a significant deterioration of cell-mediated immunity. The terminal stage of

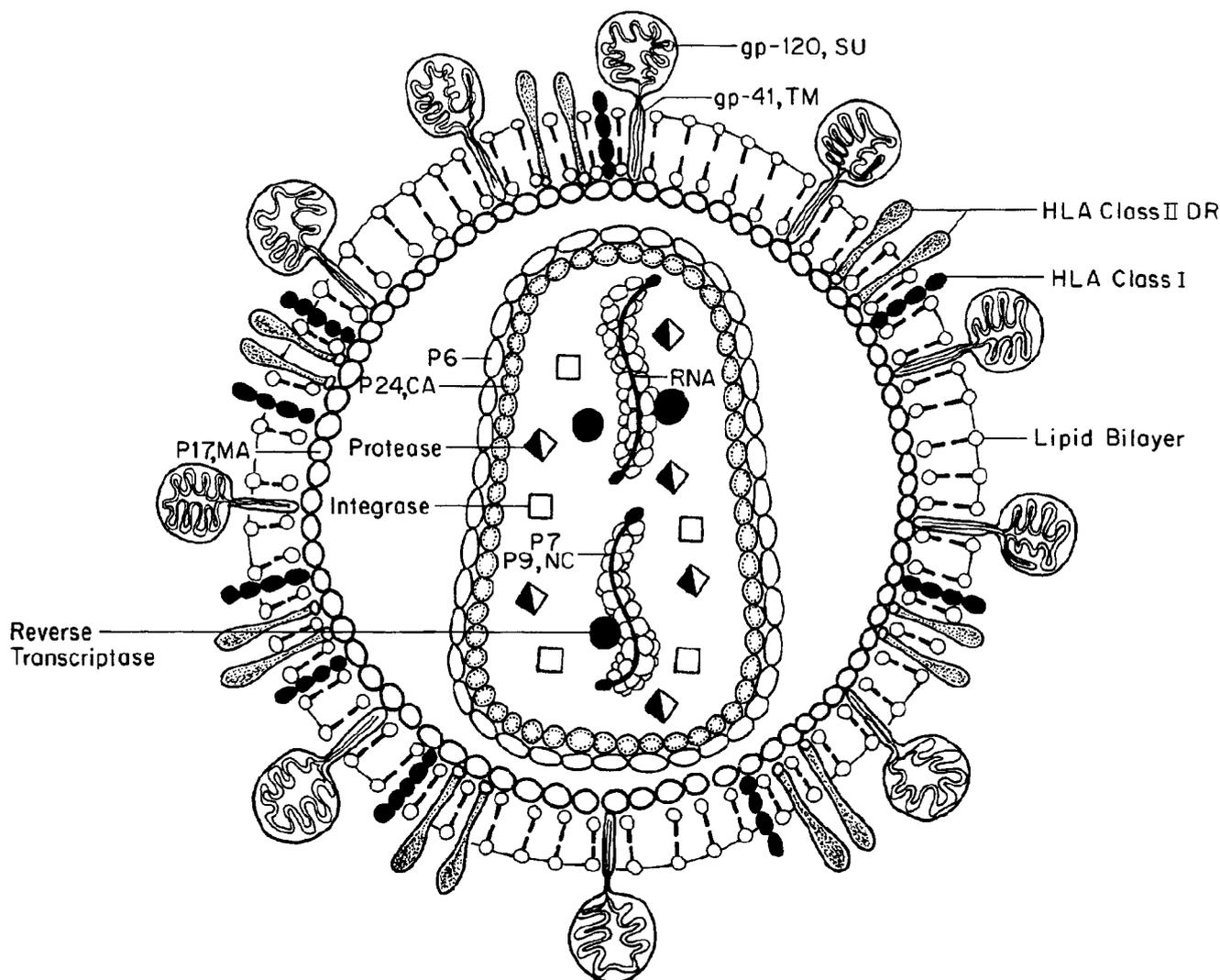


FIG. 1. HIV-1 virion. The mature HIV-1 virion measures approximately 1,000 Å in diameter. The HIV-1 genome consists of two identical, noncomplementary sense (+) strands of RNA, each approximately 9.2 kb long, packaged in a cone-shaped, protein core composed of 24 kDa capsid (CA) proteins (p24), surrounded by a unique protein, p6, that regulates release of the virus from the cell. This, in turn, is surrounded by p17 matrix (MA) proteins, and the entire virion is enclosed in a phospholipid bilayer envelope derived from the host cell membrane. The viral enzymes, RT, integrase, and protease, are packaged with the RNA in the core. The surface (SU) and transmembrane (TM) glycoproteins, gp120 and gp41, respectively, form a noncovalent complex. Also incorporated into the envelope are various host proteins including HLA Class I and II DR molecules.

HIV infection is marked by a complete collapse of the immune system. This is accompanied by various AIDS-defining illnesses, including constitutional signs and symptoms, cachexia, dementia, a variety of opportunistic infections, and/or neoplasia. Total disruption of the germinal centers and complete disintegration of the follicular dendritic cells of the lymph nodes and the release of free virions into the circulation may be seen at this point.

**Apoptosis.** Earlier studies implicated a variety of mechanisms in the progressive depletion of the absolute number of CD4<sup>+</sup> cells in the peripheral blood of patients with HIV infection. These included syncytium formation between infected and uninfected CD4<sup>+</sup> cells, selective infection or destruction of memory T cells, killing of uninfected cells by an autoimmune mechanism, Fc receptor-mediated antibody-dependent cellular cytotoxicity, and cell-mediated destruction of HIV-infected cells by cytotoxic T lymphocytes and/or NK cells. More recently another mechanism, programmed cell death or

apoptosis, has been proposed to account for some of the CD4<sup>+</sup> cell depletion observed in the peripheral blood of AIDS patients (27). Apoptosis is a physiological, cellular suicide mechanism in which cell death occurs naturally during the maturation of specific tissues or organs. Apoptosis is characterized by several morphologic and biochemical features including cell shrinkage, condensation of chromatin, and cleavage of DNA into oligonucleosome-sized (180- to 200-bp) fragments. These oligonucleosome fragments are the basis of the so-called DNA ladder formation on agarose gel electrophoresis that is a characteristic of apoptosis. Apoptosis is not restricted to only CD4<sup>+</sup> cells in HIV infection: CD8<sup>+</sup> T lymphocytes and B lymphocytes also undergo apoptosis (41). In HIV infection, apoptosis can be activated directly or indirectly. In indirect killing, CD4 molecules on uninfected cells can be cross-linked with gp120 of the virus envelope. This can prime the cell to undergo DNA fragmentation and subsequent programmed cell death when it encounters major histocompatibility complex

## HIV-1 Genes and Their Locations

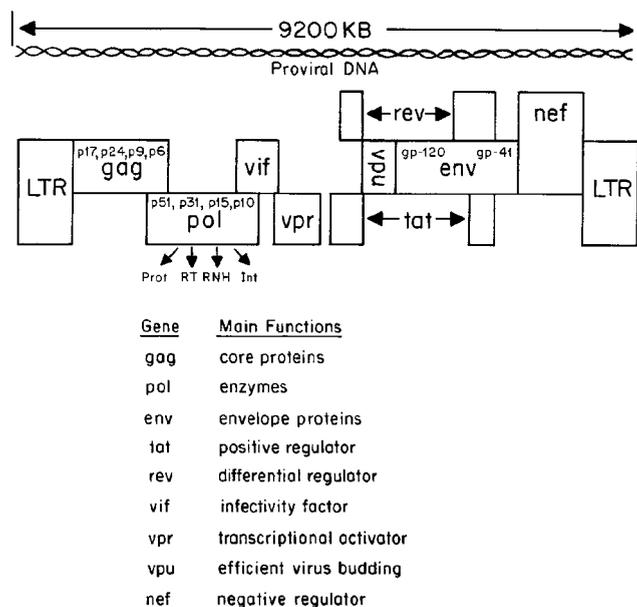


FIG. 2. Genetic map of HIV-1. Nine genes comprise the HIV-1 genome. In the proviral state the viral DNA, reverse transcribed from the viral RNA and integrated into the host's chromosomal DNA, is flanked on both ends by a stretch of sequences called the long terminal repeat (LTR). Binding of different regulatory proteins to the LTR can positively or negatively regulate transcription of virus. All retroviruses, including HIV-1, share three major genes: *gag*, *pol*, and *env*. The *gag* (group-specific antigen) gene encodes the structural proteins for the core, while the *env* (envelope) gene encodes the large (160-kDa) precursor viral glycoprotein, gp160, that is posttranslationally cleaved into gp120 and gp41. The *pol* (polymerase) gene encodes several enzymes, including RT, involved in viral replication and integration. The proteins encoded by the remaining six genes, *tat*, *rev*, *vif*, *vpr*, *vpu*, and *nef*, regulate virtually every step of viral replication. The *tat* gene product is a positive regulator of transcription while the *rev* product is a regulator of structural gene expression. The *vif* gene encodes infectivity proteins, allowing transport of the virus to the cell nucleus and stabilization of DNA intermediates. The *vpr* gene encodes viral protein R, a positive regulator of early viral transcription. Protein U, encoded by the *vpu* gene, downregulates the CD4 receptor and promotes the release of virions from infected cells. One of the first regulatory genes identified, *nef*, initially was thought to negatively regulate virus replication, hence its designation, negative factor. More recently, *nef* was shown to downregulate CD4 receptors, augment viral replication in vitro and in vivo, and promote infectivity by cell-free virus.

(MHC) class II molecules on homologous cells or superantigens derived from various microorganisms. Regarding direct induction of apoptosis, HIV-infected cells can induce this process in uninfected cells. The activation of various genes, including *fas/ApoI* (CD95), *Bcl-2*, several protooncogenes, and tumor suppressor genes, and cytokines and transcription factors are involved in apoptosis. In HIV infection, apoptosis regulated by the expression of *fas-Bcl-2* has been associated with the depletion of the CD45RO<sup>+</sup> T cell subset (8).

**T-helper lymphocyte subpopulations.** Several functional subpopulations of CD4<sup>+</sup> T-helper (Th) lymphocytes have been described including Th-1, Th-2, CD45RA<sup>+</sup> (naive), and CD45RO<sup>+</sup> (memory) cells. Activated Th-1 cells produce cytokines, such as gamma interferon, IL-2, and IL-12, that facilitate cell-mediated immunity. However, cytokines produced by Th-2 cells, such as IL-4, IL-5, IL-6, and IL-10, enhance humoral immune responses. Recent evidence suggests that a shift in CD4<sup>+</sup> subpopulations from Th-1 to Th-2 predominance is

associated with disease progression and clinical deterioration of HIV-infected patients (15). This hypothesis is supported by an animal model wherein mice lacking a Th-2 response resist the development of a murine form of AIDS. While this theory of Th-1 to Th-2 cell switching is not accepted by all AIDS researchers, clinical trials are under way using exogenously administered cytokines, such as IL-2, to expand the pool of CD4<sup>+</sup> T cells and their repertoire of T-cell receptors. Although CD45RA<sup>+</sup> and CD45RO<sup>+</sup> markers are downregulated in HIV-1 infection, (9) an increase in the CD45RO<sup>+</sup> subset of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells has been associated with progression of disease (5). HIV has been shown to preferentially infect the RO<sup>+</sup> (memory) subset of CD4<sup>+</sup> lymphocytes (29, 51).

**HIV receptors and chemokines.** Recognizing that the principal targets of HIV are CD4<sup>+</sup> T cells and macrophages, in 1984 researchers established that the CD4 molecule was the primary receptor for virus entry into susceptible target cells. However, additional HIV receptors have been described including galactosylceramide on oligodendrocytes and Schwann cells in the brain and epithelial cells of the intestines. Immunoglobulin Fc receptors on neutrophils, monocytes, basophils, mast cells, eosinophils, platelets, and B lymphocytes also may facilitate binding of HIV. Antibodies made against various HIV antigens can bind to infectious virions or infected cells that, in turn, are bound by cellular Fc receptors. The bound complex can then be internalized by the cells and subsequently infect them independently of the CD4 receptor. There is current evidence indicating that CD4 alone is insufficient for optimal entry and fusion of HIV. This is based on the observation that murine cells genetically manipulated to express the human CD4 receptor are not permissive for HIV infection. Further, studies have shown that HIV grown in laboratory transformed T-cell lines could infect only primary T cells and could not infect monocytes and macrophages. These variants were designated T tropic. By contrast, primary HIV isolates could infect only monocytes and macrophages but not T-cell lines and hence were called macrophage (M) tropic. In addition there are some viruses that can infect both macrophages and T cells. These variations in cell tropism appear to be due to sequence differences in the V3 region of the envelope glycoprotein, gp120. When gp120 binds to CD4, changes occur in both molecules producing a new conformation that requires additional points of attachment for effective viral entry. Thus, supplementary, species-specific cell surface factors may be necessary for optimal virus fusion with the membrane. Such non-CD4 factors have been differentially named by various investigators as second receptors, coreceptors, fusion receptors, accessory receptors, or alternate receptors (35).

Recently, considerable interest has been directed to the identification and cloning of other putative coreceptors for HIV, yielding some surprises (1, 16, 48). A member of the seven transmembrane G-protein-coupled receptor family, termed leukocyte-derived seven transmembrane domain receptor (LESTER) or alternatively called fusin was found to act as a coreceptor for T-tropic HIV strains only. When fusin was introduced into HIV-resistant mouse cells expressing human CD4, they became readily susceptible to HIV infection. Recently, this coreceptor for T-tropic strains of HIV, fusin, was found to be identical to the chemokine receptor CXCR-4. The natural ligand for CXCR-4 is the chemokine, stromal cell-derived factor 1 (SDF-1). Chemokines are chemoattractant cytokines, many of which have long been known to play a role in mediating allergic reactions. SDF-1 can block the ability of CXCR-4 to serve as a coreceptor for T-tropic strains of HIV. Furthermore HIV-infected patients with SDF-1 gene variants

## Macrophage Tropic HIV-1 Strain

## T Cell Tropic HIV-1 Strain

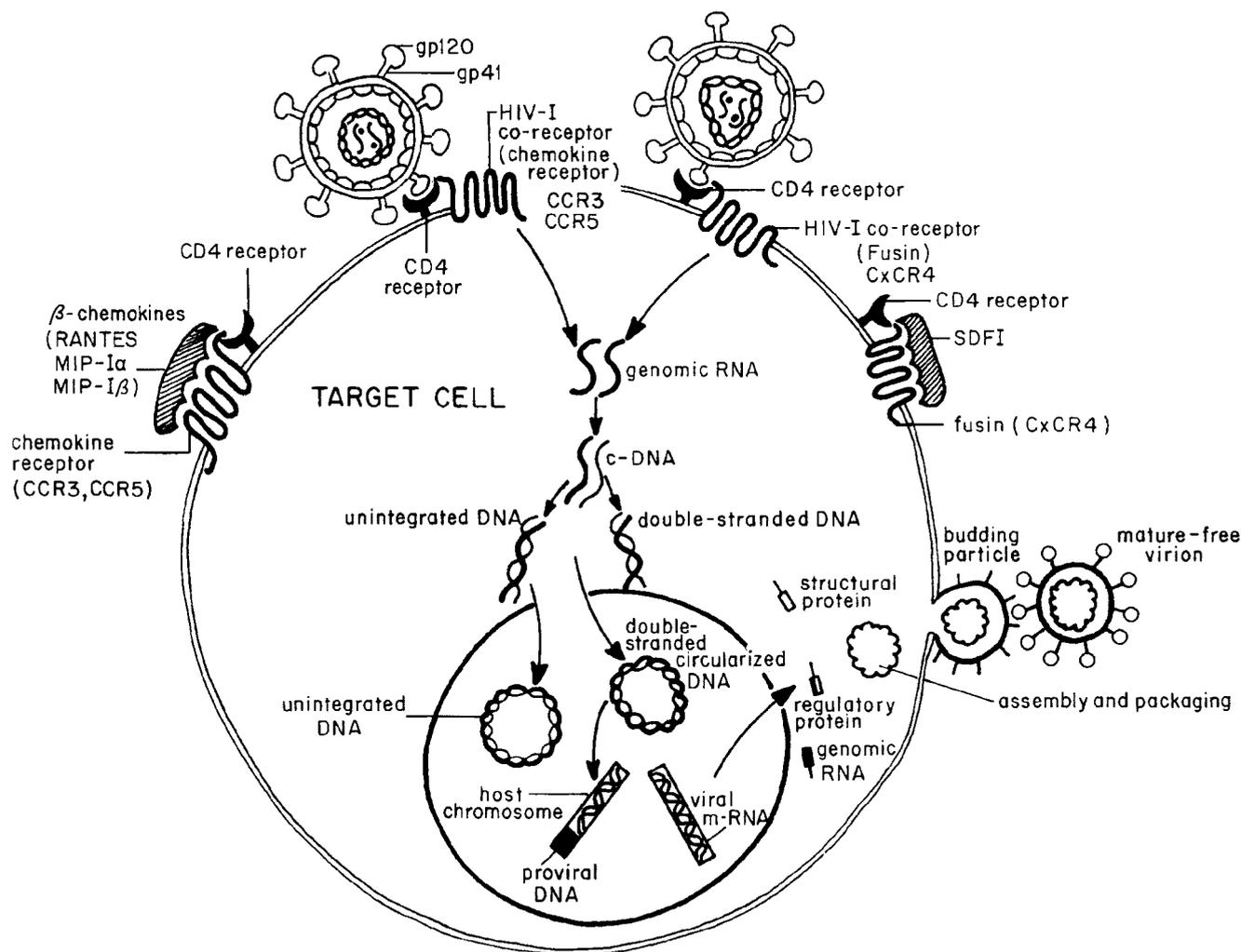


FIG. 3. Life cycle of HIV-1. In addition to CD4, the primary HIV-1 receptor on the surface of susceptible cells, fusion and entry of virus is also facilitated by coreceptors. The coreceptor for the T-cell-tropic laboratory strain of HIV-1 is a G-protein-coupled protein called fusin: it also is the CXCR4 chemokine receptor. Primary M-tropic HIV-1 clinical isolates use the chemokine receptors CCR3 and CCR5 as coreceptors. The natural ligands for T- and M-tropic viruses are SDF-1 and the β-chemokines, respectively. By competing for the virus coreceptors, the chemokines block efficient entry of the virus into susceptible cells. After HIV-1 enters the cell, the genomic RNA is reverse transcribed into double-stranded DNA. The DNA is circularized and integrated into the host's genome as a latent provirus. When the latently infected cell is immunologically activated, transcription occurs followed by viral protein synthesis. After intracellular assembly, mature virions are budded from the cell membrane causing lysis of the infected cell.

were found to have a delay in the onset of AIDS (55). CXCR-4 was also shown to be the primary receptor for HIV-2. To reflect their coreceptor requirement, the T-cell tropic virus strains that require CXCR-4 have recently been renamed X4 viruses (6).

The subsequent discovery of a coreceptor specific for M-tropic virus strains was not unexpected. This M-tropic HIV coreceptor was recognized to be the previously described chemokine receptor CCR5. Therefore, the M-tropic virus strains that require the CCR5 receptor for entry have been named R5 viruses (6). Other chemokine receptors, such as CCR2b and CCR3, also can serve as coreceptors for some HIV strains. The β-chemokines RANTES (regulated upon activation, normal T-cell expressed and secreted), and MIP-1α (macrophage inflammatory protein-1α) and MIP-1β, are ligands for CCR5.

CCR5 receptor binding by these chemokines inhibited M-tropic virus infection of CD4<sup>+</sup> cells (17). RANTES is the most active inhibitor of HIV replication. Moreover, the absence of CCR5 on a cell has been associated with resistance to HIV infection. The role of CCR5 as coreceptor for HIV in vivo is supported further by a report of resistance to HIV infection with R5 viruses by individuals who are homozygous for a 32-bp CCR5 gene deletion (Δ32 CCR5) (19). Independent confirmation of the role of chemokine receptors as coreceptors for HIV derives from previous studies showing that HIV replication in CD4<sup>+</sup> cells could be suppressed by soluble factors secreted from CD8<sup>+</sup> cells. There is a current controversy over whether these HIV suppressor factors are the β-chemokines RANTES, MIP-1α, and MIP-1β or are a unique HIV inhibitor (37). Although monocytes, B lymphocytes, mast cells, and fibro-

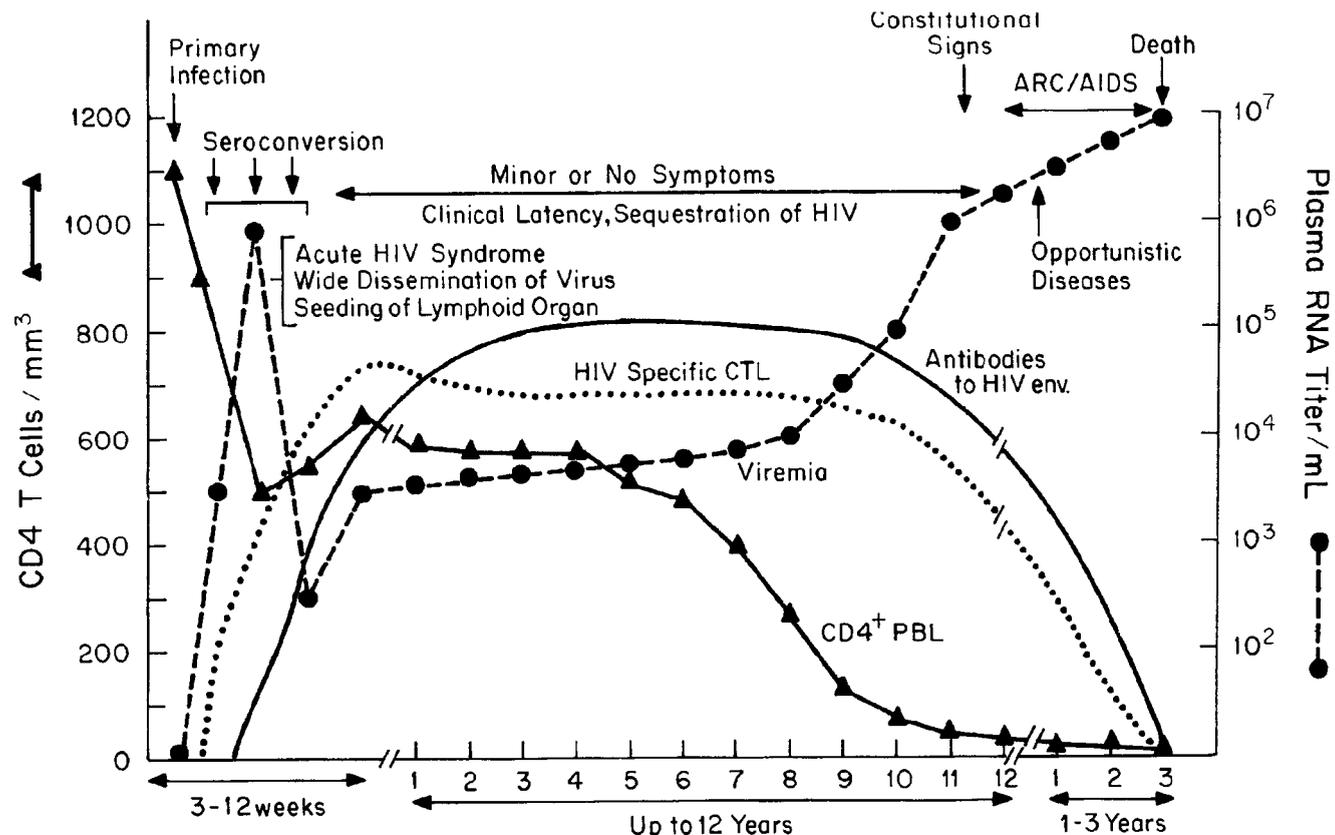


FIG. 4. Natural history of HIV-1 infection. Although reliable clinical data are available for only the past 12 years, it is predicted that approximately 95% of HIV-1-infected patients will progressively develop AIDS within 15 years of infection. The first few weeks after primary infection are characterized by an acute mononucleosis or flu-like illness. During this stage seroconversion usually occurs and is associated with a rapid increase in circulating viral titers. This is followed by virus dissemination to lymphoid organs. Adaptive host immunity, including neutralizing antibodies and cytotoxic T lymphocytes (CTL) against virus-infected cells, subsequently develops. While these responses may temporarily control the infection, generally they are unable to eliminate the virus. The patient then enters the stage of clinical latency or the asymptomatic period. A progressive decrease in CD4<sup>+</sup> peripheral blood lymphocytes (PBL) continues during the latency stage. When CD4 counts fall below 200/mm<sup>3</sup>, the disease usually enters a symptomatic phase, characterized by opportunistic infections and other AIDS-defining conditions. (Modified from reference 43.)

blasts produce  $\beta$ -chemokines in low amounts, activated CD8<sup>+</sup> cells are the most potent producers of them (18, 36). The role of the  $\beta$ -chemokines in HIV infection has become even more complicated in view of a recent report showing that they stimulate HIV replication in macrophage cultures (48). While there may be discrepancies between the various reports on the effect of  $\beta$ -chemokines on HIV, it is evident that they have significant regulatory effects on virus replication. Further studies are necessary to elucidate the specific mechanisms underlying these observations. Perhaps they will lead to new therapeutic strategies using inexpensive synthetic blockers of the chemokine receptors or ways to regulate the natural production of chemokines in vivo. Lastly, there is evidence for another cellular HIV fusion domain that binds to envelope gp41 permitting entry of the viral core into host cells. A recent study also identified proteins secreted by CD8<sup>+</sup> from AIDS patients that could block the infection of peripheral blood mononuclear cells by both M-tropic and T-tropic isolates (42).

#### DIAGNOSIS

Serodiagnosis remains the primary method for detecting established HIV infections. Screening is performed by the enzyme-linked immunosorbent assay (ELISA). This technique depends upon antibodies from a serum sample to react with a

crude extract of HIV particles. The ELISA technique has a sensitivity in the range of 93.4 to 99.8% and a specificity of 99.2 to 99.8%. If the initial ELISA result is positive the assay is repeated. After two successive positive ELISA results, the serum sample is then tested by the Western immunoblot technique. The Western blot technique detects antibodies to purified HIV proteins separated by electrophoresis; however, more recent versions of this method utilize recombinant HIV proteins. Specific antibodies binding to p24 plus at least one of the envelope proteins, gp120, gp41, or gp160, confirm seropositivity to HIV. Since the infected host takes at least 22 to 27 days to develop antibodies to HIV after infection, ELISA assays may not be able to detect acute HIV infection.

Other methods are also available for diagnosing HIV infections. The p24 antigen capture method is an immunoassay for the viral core protein. In vitro coculture of HIV-infected cells with susceptible cells also can be used to demonstrate infection. This method generally has been superseded by PCR. PCR is a powerful biological amplification technique that is capable of identifying very low copy numbers of proviral DNA in a complex mixture and exponentially expanding them to a level at which they can be detected readily. A variation on this technique can be used for detecting viral RNA, which is first reverse transcribed to DNA and then amplified by PCR. However the branched-DNA signal amplification assay is currently

the method of choice to detect and quantitate viral RNA (viral load) in plasma. Viral load, rather than CD4 numbers, is now considered to be a better surrogate marker for prognosis of HIV infection (39).

Diagnosis of neonates born to HIV-seropositive mothers poses a special problem. As maternal immunoglobulin G (IgG) is transferred across the placenta during the last trimester of pregnancy, infants born to seropositive mothers also will be seropositive irrespective of their state of infection. Maternal antibodies can remain in the circulation of the infant for up to 1 year and consequently will confound serodiagnosis of the child. A positive assay for HIV p24 in the serum of a neonate is consistent with active infection. However the p24 antigen capture assay lacks sensitivity, and false-negative assay results are not uncommon. Thus the HIV DNA PCR assay is preferred for detecting viral genomes in neonates. IgA is not transplacentally transferred, and detection of IgA antibodies to HIV proteins in the serum of neonates and infants correlates with infection and seroconversion. Coculture of neonatal lymphocytes with susceptible target cells has been used for detection of active infections, but this method is no longer popular due the difficulties and biohazards of HIV culture.

Several commercial kits have been released for home testing of HIV infection. Considerable controversy has surrounded this issue. Clearly home testing is very convenient and appeals to privacy concerns. However, critics have argued that the emphasis on privacy may perpetuate the stigma of AIDS and that the quality of test-associated counseling may not be as good as personal counseling obtained directly from a health care professional. Another concern was for the high cost of home testing that may put it out of the reach of persons at greatest risk. Recently, one manufacturer withdrew its home testing kit because of a poor consumer response. As noted above, home testing which depends upon the assay of antibodies may not detect very early acute infections.

### CLINICAL MANIFESTATIONS

Understandably it is difficult to identify patients acutely infected with HIV. However, the available information indicates that primary infection with HIV mimics more benign viral syndromes, particularly mononucleosis (43). During acute infection virus predominance may shift from the blood and localize in lymphoid tissues where extremely high rates of virus replication occur (44). While significant viral titers can be detected in circulation throughout the course of HIV infection in untreated individuals, increasing plasma viral titers during the late stages of disease may be an indicator of a poor prognosis. The clinical course of an HIV infection is depicted in Fig. 4. After primary infection there is a quiescent period during which the patient may be seronegative yet can be highly infectious. Ultimately antibodies are made to various HIV proteins and the patient becomes seropositive within 3 to 12 weeks after initial infection. However, it has been demonstrated that in one individual there was a lag of up to 39 months between initial infection and seroconversion. While this is an exception to the usual course of seroconversion, it demonstrates that it is possible for an individual to be infectious while remaining seronegative for a relatively long time.

Previously, the absolute CD4<sup>+</sup> lymphocyte count was the primary marker for the clinical staging and prognosis of HIV infections (11). Currently viral load, as a function of plasma HIV-1 RNA levels (see above), is the preferred surrogate marker (39). The viral load at the time of seroconversion is not predictive of outcome, but the viral load after 3 to 6 months of infection is a good indicator of disease progression. Also, there

is generally an inverse relationship between viral load and the absolute number of CD4<sup>+</sup> lymphocytes (11). However, since some patients show increased CD4<sup>+</sup> T-cell counts despite elevated viral load (34), measurement of both CD4 count and viral load is recommended to assess clinical status. During the asymptomatic phase of HIV infection, the total peripheral count tends to remain near normal. Paradoxically, the number of infected cells in the peripheral circulation is relatively low. This is due to a progressive homing of infected CD4<sup>+</sup> cells to the lymph nodes, resulting in disruption of their germinal centers and disintegration of follicular dendritic cells. Within the lymph nodes infection may also spread to uninfected CD4<sup>+</sup> cells, further increasing the local viral load. Thus the lymph nodes, where viral replication and numbers reach very high values, serve as major reservoirs for HIV.

As the infection progresses, persistent generalized lymphadenopathy may develop. Concomitant with evidence of immunodeficiency, such as delayed-type hypersensitivity skin test anergy, decreased in vitro T-lymphocyte proliferative responses to mitogens and antigens, and/or decreased NK cell activities (49), there may be an increase in conditions that are suggestive of a defect in cell-mediated immunity such as constitutional symptoms, bacillary angiomatosis, candidiasis, thrombocytopenia, oral hairy leukoplakia, and peripheral neuropathy, etc. With further disease progression there is an associated increase in the incidence of opportunistic infections especially *Pneumocystis carinii* pneumonitis. In addition, specific malignancies, such as non-Hodgkin's lymphoma and Kaposi's sarcoma, may develop. The latter is a malignant proliferation of vascular endothelial cells which, prior to the HIV epidemic, occurred primarily in men over 60 years old from the Mediterranean region. Kaposi's sarcoma is associated with human herpesvirus (HHV) 8 infections and may develop more readily in HIV-infected homosexual men than in other AIDS risk groups (30). A list of AIDS-defining conditions is presented in Table 2.

Encephalopathy is a serious complication of HIV infections. The AIDS dementia complex generally develops late in the course of infection in adults and then progresses unremittingly to death. It is characterized by cognitive, motor, and behavioral abnormalities. Biopsy and autopsy studies describe a wide range of cerebral lesions associated with AIDS encephalopathy ultimately producing cortical atrophy. Multinucleated giant cells are histopathologic correlates of the AIDS dementia complex, and vacuolar myelin damage may affect the hemispheric and interhemispheric white matter. Several virus-mediated neurological disorders have been reported in severely immunocompromised HIV-1-infected patients. Progressive multifocal leukoencephalopathy has been associated with the human polyoma JC virus. Primary central nervous system (CNS) lymphoma may develop secondary to infections with Epstein-Barr virus and/or Kaposi's sarcoma-associated herpesvirus, also known as HHV8. Other causes of HIV-1-associated CNS disease include (i) opportunistic infections particularly due to *Toxoplasma gondii*, herpesviruses, adenovirus, fungi, and mycobacteria and (ii) unusual primary malignancies, generally non-Hodgkin's lymphomas but non-lymphomatous neoplasms such as astrocytomas, oligodendrogliomas, and ependymomas have also been reported.

**Pediatric considerations.** Many of the general clinical manifestations described above are common to adults and children. However, there are several problems that are unique to the pediatric population. Like adults, children frequently develop pneumonitis caused by *P. carinii*. However they also are susceptible to an idiopathic lymphoid interstitial pneumonitis (LIP) that resembles *P. carinii* pneumonitis both clinically and

TABLE 2. Conditions included in the 1993 CDC AIDS surveillance case definition

Condition
Candidiasis of bronchi, trachea
Candidiasis, esophageal
Cervical cancer, invasive
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes)
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV-related
Herpes simplex, chronic ulcer(s) (>1 months duration), bronchitis, pneumonitis, or esophagitis
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi's sarcoma
Lymphoma, Burkitt's (or equivalent term)
Lymphoma, primary, of brain
<i>Mycobacterium avium</i> complex or <i>Mycobacterium kansasii</i> , disseminated or extrapulmonary
<i>Mycobacterium tuberculosis</i> , any site (pulmonary or extrapulmonary)
<i>Pneumocystis carinii</i> pneumonia
Pneumonia, recurrent
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain
Wasting syndrome due to HIV

radiographically. As therapy for these two entities is very different and often mutually exclusive, definitive diagnosis of LIP usually requires a lung biopsy. Therapy for *Pneumocystis* pneumonia includes trimethoprim plus sulfamethaxazole or pentamidine, whereas idiopathic LIP is usually treated with systemic corticosteroids. Other clinical complications of HIV infections that are unique to children include failure to thrive, renal tubular acidosis, and a dysmorphology that is controversial.

In contrast to that in adults, encephalopathy in infants may be an early marker of AIDS. It usually presents as a failure to achieve normal, developmental, motor milestones. Early treatment with antiviral drugs such as zidovudine (ZDV) may completely reverse these symptoms, suggesting that the initial encephalopathy in infants may be due to transient neurotoxins or host factors such as cytokines. In support of this hypothesis is the observation that in spite of profound neurobehavioral symptoms, virus load within the brain generally is relatively low. Furthermore gp120 from the viral envelope was shown to be neurotoxic in vitro. With disease progression cortical atrophy and ventricular dilatation occurs, ultimately producing profound decreases in brain weight. Angiopathy with dystrophic calcification of blood vessels, particularly in the basal ganglia and pons, has been described.

### THERAPY

In addition to the primary therapy described below other medical management of HIV infection includes the use of antibiotics, antivirals, and antifungals for secondary or opportunistic infections, vaccination against other etiologic agents, prophylaxis of *Pneumocystis* pneumonia, and psychological support. Although treatment of HIV-infected patients with recombinant (r)IL-2 or polyethylene glycol-modified rIL-2, alone or in combination with antiretroviral therapy, has produced some transient, beneficial changes in immunologic func-

tions and CD4 counts, no substantial decrease in viral burden has been associated with IL-2 therapy (25).

Primary therapy for HIV infection involves combinations of various antiretroviral drugs. Early antiviral therapy of HIV infection is likely to be beneficial even in asymptomatic subjects. The mainstay of HIV treatment has been the use of RT inhibitors. RT inhibitors prevent the virus-derived enzyme, RT, from synthesizing viral DNA from the HIV genomic RNA template, thus preventing integration of subsequent viral cDNA into the host's genomic DNA. While RT inhibitors may hinder normal, cellular DNA synthesis, they are much more active against viral RT. ZDV (Retrovir), formerly called azidothymidine (AZT), is the prototypic RT inhibitor. It is an analog of thymidine, a natural precursor of DNA. Other nucleoside analogue RT inhibitors include didanosine (Videx), lamivudine (EpiVir), stavudine (Zerit), and zalcitabine (HIVID). Efavirinz (Sustiva), delaviridine mesylate (Rescriptor) and nevirapine (Viramune) are nonnucleoside RT inhibitors (10).

Another class of antiretroviral drugs, the protease inhibitors such as indinavir (Crixivan), ritonavir (Norvir), nelfinavir (Viracept), and saquinavir (Fortovase and Invirase) are quite effective (10). These drugs block critical steps in virus assembly that are dependent upon proteolytic cleavage. The larger-molecular-weight gag-pol precursor gene product is post-translationally cleaved by viral proteases into the individual proteins that compose the nucleocapsid core (gag, p55, p24, p17, p15, p9, and p7) and the polymerases (pol) of HIV-1. However, the envelope precursor, gp160, is cleaved into gp120 and gp41 by host cell proteases. Synthetic inhibitors of the HIV and host cell proteases thus prevent the assembly of complete, infectious virions resulting in morphologically abnormal, noninfectious particles. Additions to the pharmacopoeia of antiviral drugs occur quite frequently.

Single-drug treatment or monotherapy against HIV infection is not effective because of the rapid emergence of drug-resistant viral mutants. Multidrug, combination therapy currently is the strategy for HIV management. However, there is no definitive evidence that multidrug therapy can completely eliminate the virus. While there have been many observations and reports of treated patients who have dropped their viral loads below the level of detection, their long-term prognosis remains guarded (3). Regarding initial therapy, two major factors must be considered: the viral load and the commitment of the patient to rigid adherence to lifelong treatment. Initiation of therapy should be considered for patients with HIV RNA levels greater than 5,000 to 10,000 copies/ml regardless of CD4 count. It is currently recommended that initial treatment consist of a three-drug regimen including two different nucleoside analog RT inhibitors (NRTI) plus a potent protease inhibitor (10). A combination of two different NRTIs plus a nonnucleoside RT inhibitor (NNTI) is an acceptable alternative. Increasing HIV RNA levels suggest treatment failure and are an indication for changing therapy. Other reasons for considering changing therapy include toxicity, intolerance, and noncompliance. When switching therapy at least two and preferably all three drugs should be changed. Single drug changes are strongly discouraged. A wide range of alternative combinations have been proposed (10). Limited CD4<sup>+</sup> T-cell increases have been reported for the highly active antiretroviral therapy (HAART) in early HIV-1 infections (23). Although HAART regimens are generally well tolerated by HIV-infected patients, several adverse effects have been described including altered body habitus, hypoglycemia, and hyperlipidemia.

Pregnancy in an HIV-infected woman poses a unique therapeutic problem as the needs of both the fetus and the mother must be considered in planning therapy. The rate of vertical

transmission of HIV from an infected mother to her child is approximately 20% in the untreated state in developed countries. A National Institutes of Health-supported clinical trial (ACTG 076) demonstrated that treatment of HIV-infected, pregnant women with ZDV commencing at 14 weeks of gestation can significantly reduce the rate of vertical transmission to the infant to about 8%. Only relatively short-term safety data is available from this trial. The sole form of infant toxicity observed was a mild, transient anemia that resolved by post-natal week 12. No congenital anomalies were observed. Regarding other retrovirals, there have been reports of premature deliveries by pregnant women treated with drug combinations including indinavir or zidovudine. However, it is not possible to determine whether this specifically was due to the protease inhibitors or to the combination used. Because of the lack of sufficient data, caution should be exercised when considering protease inhibitors during pregnancy. In any treatment plan it is essential that the risks and benefits to both mother and fetus are explained so that an informed decision can be made.

In children, treatment with intravenous IG (IVIG) was shown to reduce the morbidity but not the overall mortality of HIV infections by decreasing the severity and frequency of opportunistic infections (40). However, this benefit was limited to a subgroup of children who were not receiving trimethoprim-sulfamethoxazole for *Pneumocystis* prophylaxis (50). Recently, a clinical trial of IVIG in HIV-infected adults also showed that the frequency of serious infections and concomitant hospitalizations were significantly decreased. However, while IVIG was shown to reduce the incidence of secondary infections associated with HIV-1 disease in both adults and children (56), further studies are needed to assess the possibility of antibody-mediated enhancement of HIV-1 infections via Fc and complement receptors on the surface of cells. This could be a mechanism for infection of other target cells in addition to CD4<sup>+</sup> cells.

### PREVENTION

As HIV infection is an STD, a vigorous educational program to promote safe sex practices is paramount to reducing transmission of HIV (4). Barrier protection with latex condoms is helpful. There has been speculation that the spermicide nonoxyl 9 may have anti-HIV activity, but this has not been substantiated in a controlled clinical trial. Unprotected, receptive anal intercourse among homosexual men is a high-risk activity. Screening of blood donors for HIV antibodies and donated blood and derivative products for the p24 core antigen has improved the safety of the blood supply. There is a "window period" of approximately 22 to 25 days after initial infection before detectable seroconversion occurs. The p24 antigen capture assay was proposed to shorten this window period by 5 to 10 days. However, it lacks sensitivity to low levels of antigen and antigenemia often is transient. When antibodies to p24 are present, they can mask detection of antigen by forming immune complexes. The p24 antigen assay was unable to detect up to 75% of seronegative blood donors who were infected (26), and its mandated use adds a major cost to processing donated blood. Thus, additional methods such as heat and detergent treatment, increased purification, and the use of recombinant materials (e.g., rFactor VIII), etc. are needed to make blood products safer. HIV infection among intravenous drug users and their sexual partners has been increasing steadily. Although politically controversial, there is evidence that the ready availability of sterile hypodermic needles and syringes can reduce HIV transmission. This has been attempted through exchange programs or by eliminating the

need for prescriptions to purchase syringes and hypodermic needles from pharmacies.

Clearly health care workers are at increased risk of exposure to HIV. Because of this occupational hazard, public health authorities have implemented the practice of universal body fluid precautions. Thus, all body fluids from all patients should be handled as if they contained infectious virus. Skin with open lesions and mucous membranes are particularly vulnerable. Hence the use of latex gloves and face shields is strongly recommended to reduce the risk of occupational exposure. Recapping of hypodermic needles presents a high risk for injury and this habit must cease. All contaminated needles and sharp instruments should be placed directly into a secure, closed container for subsequent sterilization.

An earlier epidemiological investigation of health care workers by the CDC demonstrated that the risk of seroconversion after percutaneous exposure (e.g., needle stick or laceration) to a known infected source was about 0.5%. The average risk of HIV infection from all types of exposure to infected blood is only 0.3%. Even though these are remarkably low rates, the consequences of HIV infection are so serious that prevention is the best option. Several studies have demonstrated a potential benefit from postexposure chemoprophylaxis of HIV infections (45). Thus, the CDC developed guidelines for a 4-week course of chemoprophylaxis in the event of an occupational exposure to HIV. Prophylaxis ranges from single-drug treatment with ZDV alone to a three-drug regimen in cases of exposure with the highest risk. Exposed individuals should be tested for seroconversion at 6 and 12 weeks and at 6 months and should be counseled about the possibility of secondary transmission. If chemoprophylaxis is instituted, persons should be evaluated for drug toxicity by monitoring complete blood counts and by performing renal and hepatic functional assays. Prophylaxis of opportunistic infections should also be considered along with antiretroviral prophylaxis for certain high-risk groups.

**Vaccines.** In any virus infection, the guiding principle for an effective vaccine strategy derives from the natural immunity which is part of the recovery process. In addition to stimulating protective antibodies against HIV the ideal vaccine should also induce specific cytotoxic T lymphocytes against HIV-infected cells and activate nonspecific NK cells. In experimental systems, HIV can induce vigorous humoral and cell-mediated immune responses that can effectively neutralize the virus and lyse infected cells. However, in the clinical situation, host immune responses do not seem adequate to eliminate HIV infections. This places HIV in the category of viruses against which an effective vaccine has yet to be developed (21). Vaccines have been the most effective defense against other viral diseases such as polio and smallpox. Development of a potent vaccine against HIV has been complicated by a number of factors including: (i) the genomic variability of the virus, (ii) a lack of good animal models, (iii) a lack of satisfactory surrogate markers of protective immunity, (iv) the intracellular mode of HIV transmission, and (v) the persistent nature of infection. Since 80% of HIV infections occurs sexually, a good vaccine should stimulate mucosal immunity particularly in the reproductive tract. Use of live, attenuated or whole, inactivated HIV as a vaccine has advantages such as easy preparation and potential induction of long-term, natural immunity. However some concerns such as variability of virus strains, contamination of vaccine with host cell proteins, and safety in immunodeficient patients remain to be assessed. Several candidate HIV vaccines are composed of synthetic, recombinant, or highly purified subunit epitopes spanning various regions of the HIV proteins gp120, gp41, Tat, Rev, and Nef. Subunit

antigens are considered to be safe in comparison with killed whole or live-attenuated virus, but subunit vaccines are generally less immunogenic. Neutralizing antibodies have been detected against a specific epitope of gp120, the V3 loop, and this region has attracted considerable attention as a vaccine candidate. Currently various adjuvants, such as detoxified lipid A, adjuvant emulsions, liposomes, biodegradable microspheres, muramyl peptides, and saponin, are being evaluated for their ability to increase immune responses to HIV vaccines. The recent discovery of the X-ray crystallographic structure of gp120, the subsequent revelation of the gp120-CD4 interface, and identification of the conserved site for the chemokine receptor (33) may provide critically needed information to develop an effective and safe HIV vaccine.

While the search for an effective HIV vaccine remains elusive, there are some unique clinical observations that suggest there are intrinsic host protective mechanisms against the virus that can be upregulated by vaccination. Individuals who are seropositive for HIV but show no evidence of viral genome have been reported, suggesting the possibility of a spontaneous remission. Also there is a cohort of African prostitutes who are evidently at high risk but have not seroconverted nor do they harbor virus genome. All of these observations are encouraging and support the continuation of efforts to develop an HIV vaccine.

### GENE THERAPY

In spite of the advances described above, a definitive cure for HIV infection remains elusive (46). This is partly due to the unique life cycle of the virus as well as its plasticity and antigenic variability. Genetic manipulation of somatic cells has recently been proposed for prophylaxis and therapy against a variety of infectious diseases including HIV. This involves the stable insertion of "resistance genes," also called intracellular immunization, into HIV-susceptible cells to render them resistant to infection. Further, the ectopic expression of genes encoding specific viral antigens that evoke antiviral immune responses is also currently being evaluated. These approaches include the use of modified transdominant HIV proteins, RNA decoys, antisense RNA, ribozymes, and cellular proteins such as soluble CD4. Plasmids containing "naked" DNA encoding various HIV-1 genes have recently been injected into animals, including nonhuman primates, and human volunteers as potential vaccines. Thus, the HIV-1 genes are expressed in vivo in the absence of infectious virus and the resultant gene products may induce immune responses by the host. Whether these new methods can protect against infection with HIV-1 remains to be determined. Nevertheless they are promising and ongoing research may yield a long-awaited breakthrough.

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