**TEACHING UNIT 12**

**PATHOGENIC HUMAN RETROVIRUSES, REVERSE TRANSCRIPTASE, HISTORY OF RETROVIROLOGY**

**KEY FACTS:**

Pathogenic retroviruses are RNA viruses that contain the enzyme reverse transcriptase, which allows them to use RNA as a template for DNA synthesis. They include the human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS), and human T lymphocyte leukemia viruses type 1 and 2 (human T lymphocyte leukemia virus, HTLV- 1 and HTLV-2), associated with the development of leukemia and lymphoma.

**Prevalence:**Since the first registered cases in the early 1980s, HIV infection has reached the proportions of a worldwide pandemic. Other human retroviruses have a limited geographic distribution and a lower tendency to cause severe disease.

**Virus entry:**HIV is transmitted through sexual contact, parenterally (through blood), intravenous drug abuse, and vertically (from mother to child).

**Expansion:**HIV binds predominantly to the CD4 molecule found on helper T lymphocytes and macrophages, enters and infects these cells.

**Multiplication:** Retroviruses can cause disease years after the initial infection. HIV has a long latent period (10 years or more).

**Tissue damage:** HIV infects the cells of the immune system, especially CD4+ T lymphocytes, and causes the progressive destruction of these cells. As a result, the immune system is suppressed, making the infected person susceptible to opportunistic infections. HIV is subject to antigenic variation.

**Diagnosis:** Screening is testing for the presence of specific antibodies to HIV (or other) retroviruses. Detection and quantification of infection is carried out by nucleic acid amplification (PCR) and Western blot techniques.

**Treatment and prevention:** Treatment of HIV infection with antiviral drugs is very effective today. Although vaccine development has been slow, recent advances in the development of microbicidal agents and the use of antiretroviral drugs in prevention have provided new approaches to reduce the spread of the epidemic.

In the last 30 years, since five previously healthy patients were diagnosed with pneumonia caused by Pneumocystis jiroveci in May 1981, AIDS has epidemiologically turned from individual cases into a pandemic. During the initial phase of the epidemic, it became apparent that the disease was caused by an infectious agent with a long incubation period that could be transmitted through sexual contact or parenterally (through the blood). By 1983, the presence of a retrovirus (subsequently described as human immunodeficiency virus type 1 or HIV-1) was associated with the onset of the syndrome, and the disease-causing agent had spread far beyond its initial epidemiologic and geographic boundaries. It is estimated that there are more than 34 million people infected with HIV in the world, about 70% in Africa and 20% in Asia. This disease has been attributed to the death of more than 30 million people in the world, and the annual death rate now reaches about two million. Effective retroviral therapy has been developed, but the infection continues to spread, especially in parts of the world where this therapy is not available. In some African countries, more than 30% of the population is infected with HIV, where it has the most devastating medical and social effects.

Two characteristics make AIDS unique among infectious diseases: it is a fatal disease, and most of its devastating symptoms are not the result of direct action by the causative agent of the disease. In conditions of suppression of the host's immune response by HIV infection, opportunistic microorganisms can cause a number of different clinical syndromes. In fact, most symptoms in people with AIDS are the result of a secondary infection rather than the virus itself.

**History of retrovirology**

Understanding the role of retroviruses as etiological agents of AIDS requires a historical review of the complex relationship between viruses and cancer. This history presents a challenge to the basic tenets of molecular biology, which hold that genetic information is transferred from DNA molecules, via RNA mediators, to proteins. In the late 1960s, researchers identified an unusual type of virus whose genetic information is written in an RNA molecule. Although RNA viruses were not new, these viruses were unique because they contained the previously unknown reverse transcriptase enzyme. Using RNA as a template, this enzyme participates in the synthesis of complementary DNA, which is eventually integrated into the genome of the host cell. This DNA, called a provirus, serves as a transitional form in the replicative cycle of the virus.

Some of these viruses (later called retroviruses because of the specific mode of replication, "in the reverse" or "retro" direction) are able to cause tumors. This type of virus was first isolated in 1911, when Peyton Rous pointed out that viruses can be the cause of tumors in chickens (later known as RSV, Rous sarcoma virus) easily transmitted through tumor extract. Since then, hundreds of retroviruses have been isolated from many groups of vertebrates. In the early 1960s, a tumor-causing virus in cats, later named feline leukemia virus, was discovered. This virus has proven important for understanding retrovirus biology for two reasons. First, he enabled the discovery of another feline retrovirus in 1986, the feline immunodeficiency virus, which causes an AIDS-like immunodeficiency syndrome in cats. Second, feline leukemia virus is transmitted among cats in the environment, providing an important model for epidemiologic studies of retroviral infections.

By the late 1960s, there was considerable skepticism about the theory that a virus mediated tumor transmission. Considering that the formation of tumors is based on genetic alterations, it was difficult to imagine how the viral RNA could interact with the DNA of the host cell in the process of oncogenic changes. The discovery of reverse transcriptase explained the mechanism of induction of permanent genetic changes. Since 1980, two groups of retroviruses capable of causing disease in humans have been isolated and described. For several years before 1980, it was suspected that retroviruses could cause disease in humans, but this could not be proven, because these viruses do not grow in cell culture. Advances in virus cultivation technology have overcome this obstacle. One of the most important was the discovery of interleukin-2, which stimulates the growth of T lymphocytes in vitro. These lymphocytes can be used to isolate human HTLV.

The first of these viruses, HTLV-1, was isolated from the cells of two patients with adult T-cell lymphoma. Later, HTLV-1 was isolated from other patients with T-lymphocytic leukemia or lymphoma, as confirmed by serological tests and nucleic acid hybridization. Epidemiological studies have shown a causal relationship between HTLV-1 virus infection in childhood and the onset of leukemia or lymphoma in several percent of infected individuals even 40 years later. A similar virus, HTLV-2, was later isolated from a patient with hairy cell leukemia, but its role in human disease is less clear. Malignancies caused by HTLV-1, T-lymphocytic leukemia and adult lymphoma, are fatal but relatively rare diseases (even in infected individuals) and are limited to certain populations. HTLV infection is also associated with some progressive diseases of the spinal cord, such as tropical spastic paraparesis and HTLV-1 associated myelopathy.

As the first human retrovirus discovered, HTLV-1 has attracted considerable attention. Although infection with this virus is rare in the US, concern about the possibility of spreading the virus through blood has led to the introduction of routine screening of blood donors for the presence of the virus. Coincidentally, research on the HTLV-1 virus provided the technology needed to isolate the causative agent of AIDS a few years later. Three years after the syndrome was first described in 1981, it was shown that the causative agent of AIDS is a retrovirus. Since it was first isolated, the virus has had several names, but today it is known as human immunodeficiency virus type 1 (HIV-1). Years later, another member of this family, HIV-2, was discovered in West Africa. This virus shares many of the characteristics of HIV-1, although it is generally less contagious. The rest of this chapter focuses on HIV-1.

**HIV – virion structure**

HIV is a small, spherical virus surrounded by a lipid envelope (Figure 1). The genome contains two identical RNA molecules, which resemble eukaryotic messenger RNA, as they contain a cap structure at the 5' end and a poly A sequence at the 3' end. Enzymes reverse transcriptase, integrase and protease are attached to the genome, which are packed into a cup-shaped core composed of capsid protein r24 and surrounded by matrix proteins r17. The nucleocapsid is surrounded by an envelope made of membrane phospholipids originating from the host cell. Membrane proteins encoded by the virus genome (gp41 and gp120) are inserted into the lipid envelope.

Four viral genes are essential for retrovirus replication (Figure 2). Gag gene encodes several basic (Gag) proteins of the viral envelope, necessary for the entry of the viral DNA into the nucleus. Pol gene encodes reverse transcriptase or RNA-dependent DNA polymerase (Pol), an enzyme responsible for genome replication, as well as integrase, an enzyme required for the integration of viral DNA into the host cell genome. Env gene encodes two viral envelope glycoproteins gp120 and gp41 necessary for binding of the virus to specific receptors and penetration into the cell. Pro gene encodes a protease necessary to cleave Gag and Pol proteins and create their active form. In addition to the genes common to all retroviruses, gag, pro, pol and env, the HIV genome contains at least six other genes. These genes encode proteins that are important in regulating the complex replication of the virus, which can persist in a latent state in an infected cell and then begin rapid replication at the appropriate time.

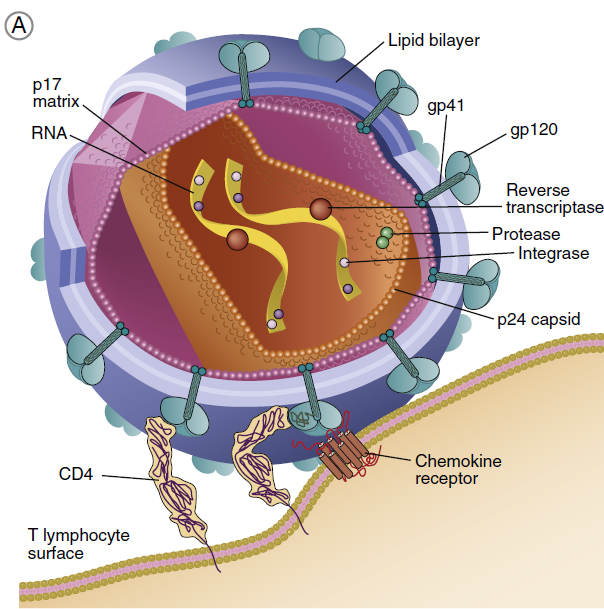
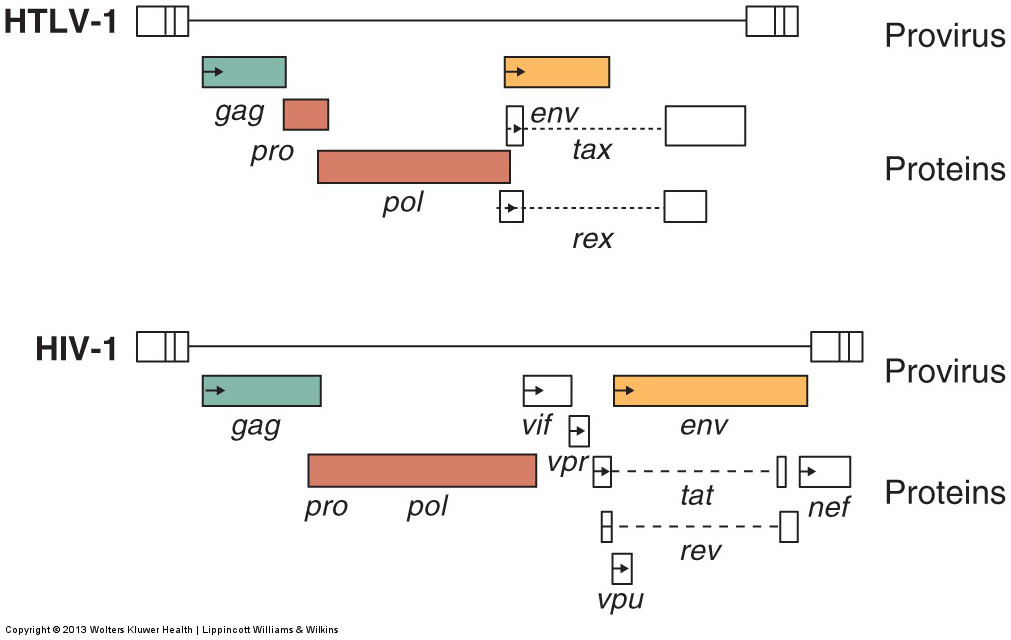
 

Figure1 Figure 2

**Prevalence and transmission of HIV**

The prevalence of HIV infection in the population is consistent with the characteristics of the virus, which is very sensitive and cannot easily enter the host's body through intact surfaces. In this sense, it resembles hepatitis B virus (but is much less contagious) and has similar epidemiological characteristics. HIV has been isolated from numerous body fluids including peripheral blood, semen, cervical secretions, milk, urine, cerebrospinal fluid, saliva and tears. The last four do not represent an important route of virus transmission. With few exceptions, HIV is mainly transmitted in three ways: through sexual contact, parenterally (via blood), and vertically from an infected mother to her child. Sexual transmission and intravenous drug abuse represent the most important routes of transmission. Other sexually transmitted diseases, especially genital ulcers, are associated with an increased risk of HIV transmission, possibly as a result of compromised skin or mucosal integrity.

Transmission of HIV through blood transfusion is very rare since routine blood screening was introduced. Hemophiliacs are at high risk for contracting blood-borne viruses, including hepatitis B and C viruses, because they receive factor VIII and IX preparations derived from the plasma of thousands of donors. Since 1984, factor VIII concentrates have been free of HIV because blood donors are tested for HIV antibodies. In addition, plasma products are treated with heat and chemicals to inactivate the virus.

Genetic factors are important determinants of susceptibility to infection. One of them is a mutation in the gene encoding CCR5, a chemokine receptor that also serves as a co-receptor for HIV. The mutated form of this gene is detected in about 10% of people of European origin, but it is found much less often in other populations. The 1% of this population who are homozygous have a very high (though not absolute) level of resistance to HIV infection. Heterozygous individuals are not protected from infection, but appear to have slower disease progression.

Vertical transmission (from mother to child transplacentally) is the most important route of transmission in pediatrics. Since the discovery that the use of antiretroviral therapy prevents transmission from mother to fetus, the number of new pediatric cases has decreased sharply in the US and Europe. In the absence of treatment, between 25 and 33% of HIV-infected women pass HIV to their children. If the mother is diagnosed with infection and antiretroviral therapy is started, the transmission rate is reduced to less than 1%.

Although HIV transmission among health care workers is an area of ​​particular concern, extensive studies have shown that HIV infection in the medical setting is rare. The risk of infection after an accidental needle stick from an HIV-infected patient is less than 0.3%. The risk of percutaneous transmission is greater if the sharp object is hollow, if the patient is in a phase of the disease when viral replication is more intense, if the sharp object was in a place where the virus is more present (for example, in a vein as opposed to a muscle), or if the injury includes the presence of a wound (instead of a simple puncture). The risk of mucous membrane exposure or contamination of intact skin is significantly lower. With the widespread adoption of "universal precautions", which imply that the blood and other body fluids of all patients are potentially infectious, and the increasing use of single-use medical devices, which are designed to reduce risk to the user, the spread of HIV among healthcare it is very rare for workers. The rate of HIV infection in the health care setting can be further reduced by prompt administration of a combination of antiviral drugs after exposure. The spread of HIV from healthcare workers to patients is even less common. Transmission of HIV in households through non-sexual contact or among work colleagues is extremely rare. Although insects as vectors stand out as a possible route of transmission, there is no evidence to support this claim.

**Entry of the virus into the body**

The mechanism by which HIV establishes infection in the host is not fully understood. HIV can enter the host via infected cells, such as macrophages, lymphocytes, or spermatozoids, or as a free viral particle. HIV can also enter the body through microabrasions on the mucosal surface, by penetrating through intact skin after a needle stick, or through intact mucosal surfaces.

**Virus spread**

Although HIV can infect many cell types, two main groups of cells in the body serve as targets for HIV infection: helper T lymphocytes and macrophages, which express the CD4 molecule and the corresponding co-receptors for HIV (chemokine receptors, CXCR4 and CCR5). These cells further transport the virus to tissues where they are normally present in large numbers (lymph nodes, spleen, blood and body fluids).

**Virus replication**

The replication cycle of HIV involves the following steps (Figure 3):

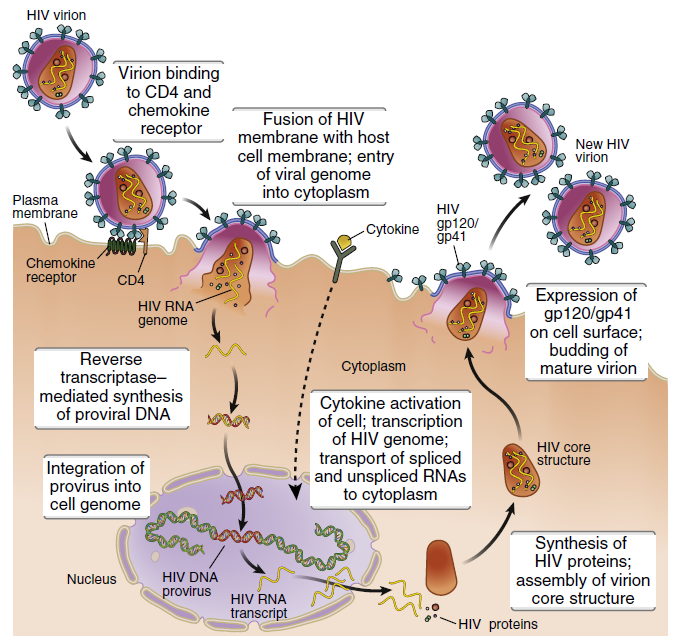


Figure 3.

• Binding (adsorption): HIV binds to the CD4 molecule via the envelope glycoprotein gp120. Antibodies specific to gp120 or cell receptors block this interaction and prevent infection. The presence of CD4 molecules on the cell surface determines which cell will be the target for infection. After binding to the CD4 molecule, gp120 of the viral envelope binds to one of two co-receptor molecules (CCR5 or CXCR4). These molecules physiologically serve as chemokine receptors, while in the case of HIV infection, they allow the virus to firmly bind to the cell membrane and induce conformational changes in the gp41 protein, which enables binding of its hydrophobic domain to the cell membrane.

• Fusion: The fusion of the viral envelope with the cell membrane is facilitated by the hydrophobic interaction between the gp41 protein and the target cell membrane. After fusion, the virus loses its integrity and characteristic morphology. The viral core, which contains genomic RNA and reverse transcriptase molecules, is released into the cytoplasm.

• DNA synthesis: Reverse transcriptase is the enzyme responsible for the synthesis of the DNA strand that is complementary to the RNA molecule of the viral genome. The enzyme then synthesizes the second strand of the DNA molecule (complementary to the first), so that a double DNA molecule is formed. In the process of DNA synthesis, the parts at the ends of the genomic RNA are duplicated, so that at each end of the newly synthesized DNA there are specific sequences called long terminal repeats (long terminal repeat, LTR).

• Integration: The double-stranded DNA molecule is then transported to the nucleus and integrated into the genome of the host cell. The enzyme responsible for the integration process is the viral genome-encoded integrase, which joins the ends of each of the LTR sequences to the DNA of the host cell. In an integrated state, the viral genetic material is called a provirus. A provirus is analogous to a cellular gene, is transmitted to daughter cells after division, and contains signal sequences to control the process of transcription into RNA.

• Synthesis of new viruses: In the "productive phase" of infection, viral DNA is transcribed into mRNA with the help of cellular DNA-dependent RNA polymerase. The signals that direct and use the cellular machinery for viral RNA synthesis are found in the LTR sequences. After transcription, some of the newly synthesized viral RNA molecules are used as mRNA for the synthesis of viral proteins, while others are incorporated into the genome of new viral particles. Virion assembly takes place on the cell surface. Structural proteins fuse with the viral genome and acquire an envelope by passing through the host cell membrane. After budding through the membrane, the viral protease cleaves the viral protein precursors and the morphological changes in the virion are completed.

• Latency and reactivation: The replication cycle described so far is common to all retroviruses. HIV and similar viruses have some additional properties. First, HIV infection includes a latent phase in which infected cells contain provirus (integrated viral DNA), but do not express viral RNA or viral proteins. Second, HIV expresses macromolecules that regulate viral genome expression and function as soluble factors. At least two HIV genes (tat and rev) have the function of transactivation factors, which strongly increase the expression of viral RNA and proteins. Tat protein (transcription activator) accelerates and enhances the transcription of integrated viral DNA with the help of host RNA polymerase, while Rev protein (regulator of viral gene expression) promotes the transport of viral RNA from the nucleus to the cytoplasm. Third, HIV proviruses contain promoters that induce the expression of viral genes when HIV-infected cells are stimulated with an antigen or infected with another microorganism. The consequences of the mentioned properties of the virus are that after infection of lymphocytes with HIV and integration of the provirus, the infectious process can be stopped and explosively activated again after a certain time by an unknown stimulus. More precisely, if T lymphocytes, macrophages or dendritic cells are activated by some external stimulus, such as an infection, the cell responds by transcribing a number of its own genes, often also by producing cytokines. An adverse consequence of such a normal protective response is the activation of the provirus, which induces the production of viral RNA and proteins. The result is explosive virus production and rapid death of the infected cell. Although only a small number of infected cells enter a latent state, these cells prevent effective eradication of the virus by antiviral therapy, even if it is administered for a long period of time.

In addition to the mentioned effects of Tat and Rev proteins, HIV-1 has other gene products that interact with host cell proteins in order to avoid the specific immune response and intracellular protective mechanisms that can limit viral replication. For example, the Nef protein (a negative effector) reduces the expression of MHC class I molecules on the cell surface, blocks apoptosis and enhances viral infectivity. Another viral protein, Vif protein (viral infectivity factor) reverses the inhibitory effects of cellular components. Finally, the Vpu protein (viral protein) promotes the destruction of CD4 and affects the release of virions.

**How does HIV evade the host's immune response?**

**Antigenic variations of HIV**

A unique feature of HIV infection is that the host's immune response is unable to completely limit viral replication (although it can partially do so during the latent phase of the disease). How does HIV manage to survive in the host despite an antiviral immune response? Several mechanisms may be important: viral gene products may be relatively invisible to the immune system; the virus can mask or change its antigenic repertoire; and the virus primarily replicates in lymphoid follicles, where immune system cells specific for virus antigens do not migrate freely.

Which HIV viral gene products are responsible for inducing the host's immune response? Knowledge about this was obtained by researching the genetic diversity of viral genes. Genes encoding internal viral proteins (gag and pol) show relative stability, while env gene is subject to numerous mutations resulting in variability in surface glycoproteins gp41 and gp120. Although antibodies to Gag and Pol proteins have been detected in infected individuals and are used for diagnosis, these antibodies have no role in limiting viral replication. On the other hand, some antibodies to viral envelope proteins can neutralize the virus, but their titers are very low compared to those in other chronic viral infections. HIV envelope glycoproteins have two important characteristics. First, they are extensively coated with polysaccharide chains, which reduce their immunogenicity. Second, they contain hypervariable regions that allow new antigenic configurations to be presented to the host's immune system. The sequences of gp120 surface glycoprotein involved in interactions with cellular receptors must be genetically conserved. Conserved sequences can be hidden and thus protected from neutralizing antibodies by carbohydrate chains and hypervariable regions. As a result, HIV constantly changes its surface antigenic repertoire, which allows it to evade neutralization. In this sense, HIV resembles the influenza virus and trypanosomes, that is, it avoids the host's immune response by changing large surface antigens. These mechanisms hinder the development of an effective vaccine containing surface glycoproteins.

**Tissue damage caused by HIV**

The molecular mechanisms responsible for lymphocyte destruction in HIV-infected individuals are not fully understood. However, based on the known abnormalities of humoral and cellular immunity, it is possible to outline a series of events that accompany HIV infection.

**Pathogenesis of the disease caused by HIV (Figure 4)**

During acute HIV infection, the virus infects cells that express the CD4 molecule in local mucous membranes, and then rapidly establishes infection in the draining lymph nodes. Studies in primates have shown that in most cases of sexual transmission, a limited number of viruses are involved in the first round of replication in the submucosal tissue. During the next few days, local replication is limited to cells present at the site of viral entry. In most cases, the number of susceptible cells locally decreases and the infection "stops" at the site of initial inoculation. However, cytokines and chemokines, produced as part of the primary immune response, recruit additional components of the immune system. If the recruited cells arrive at the site of inoculation after the completion of the first cycle of replication, the infection is not productive and there is no infection. On the other hand, if local viral replication is still ongoing at the time of immune system cell migration, the conditions for further viral replication are created, and the infection spreads and becomes self-sustaining. This early window of susceptibility is probably related to the relatively low level of infectivity of the virus during sexual contact, and relatively modest antiviral interventions can further reduce the risk of transmission.

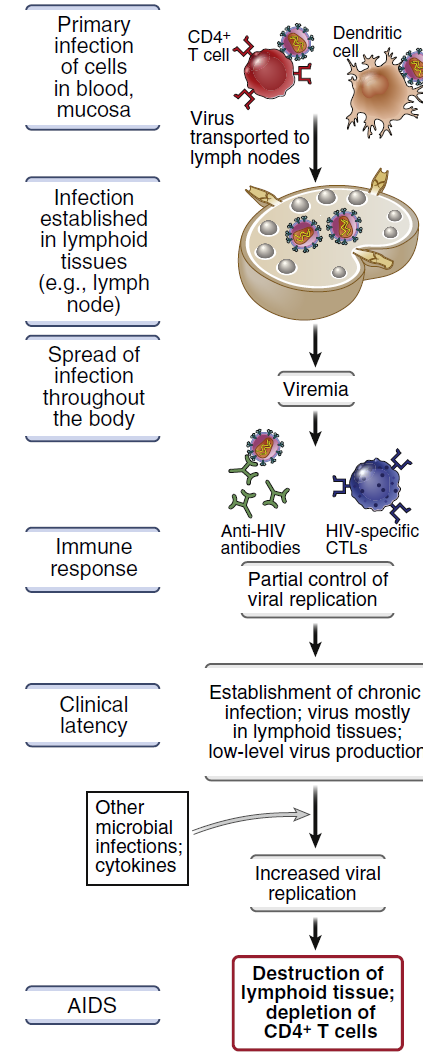


Figure 4.

After infection at the site of entry, the virus spreads rapidly systemically, to distant organs of the lymphatic tissue and the central nervous system (CNS). At this stage, the virus shows the highest level of replication throughout the course of the disease. The virus appears in genital secretions, and as in many other infectious diseases, the possibility of transmission is greatest in the initial phase of infection. Acute infection is often asymptomatic or may present with symptoms similar to acute mononucleosis, such as fever, headache, malaise, and transient rash. In the first weeks from the beginning of the infection, specific cytotoxic T lymphocytes appear in the peripheral blood and lymph tissue, and soon after, neutralizing antibodies can be detected in the plasma.

During this period of rapid viral replication, a lifelong process of generating viral diversity is initiated, and the host is faced with the challenge of developing an immune response against a rapidly changing pathogen. At the same time, the virus destroys CD4+ T lymphocytes, especially the virus-specific CD4+ T lymphocytes necessary for an effective immune response. Since the error rate of HIV reverse transcriptase is about 1 in 10,000 nucleotides, on average one new nucleotide is introduced in each replicative cycle. Given that about a billion viral replications occur in infected individuals every day, the capacity to generate viral diversity at the individual and population level is enormous.

HIV-infected cells can be directly destroyed in the process of viral replication or by effector mechanisms of a specific immune response (cytotoxic T lymphocytes or antibody-mediated cytotoxicity). After the first few months of infection, a balance is established between viral replication, effector immune mechanisms and available cells for viral replication, and the infection enters a latent phase during which the infected person is asymptomatic. After the initial phase of HIV infection, viral replication is confined mainly to the lymphatic organs where the main target is activated CD4+ T lymphocytes and it is thought that 99% of viral replication takes place in them. The remaining 1% of viral replication occurs in monocytes and the remaining CD4+ T lymphocytes, which serve as sites for the establishment of viral latency.

The loss of the CD4+ T lymphocyte population is accompanied by progressive immunodeficiency, which ultimately results in the appearance of opportunistic infections and malignancies. Although there is individual variation, the duration of the asymptomatic period before the onset of AIDS is about 10 years. During this period, the number of CD4+ T lymphocytes decreases from a normal level of about 1,000 cells/mm3 to less than 500 cells/mm3. People with advanced disease usually have less than 200 CD4+ T lymphocytes per mm3. The risk of infections is highest in the terminal stages of AIDS, when the number of CD4+ T lymphocytes decreases to less than 50 cells/mm3. Serial measurements of the number of CD4+ T lymphocytes serve to assess the risk of infections and are a guide for the implementation of antiretroviral therapy.

The rate of immune and clinical disease progression is directly dependent on the degree of viral replication, which varies considerably from individual to individual. The level of viral replication is closely related to the number of HIV RNA copies in the plasma. Individuals with high levels of circulating viral RNA (105 copies/ml or more) have a higher risk of disease progression within a few years, while infected individuals with lower levels of viral RNA (<104 copies/ml) remain asymptomatic for 10 years or longer.

In addition to reducing the number of T lymphocytes (lymphopenia), HIV also causes abnormalities in T lymphocyte function. Normally, helper T lymphocytes modulate the function of other cells of the immune system, including B lymphocytes, monocytes, and NK cells. Although many people with AIDS have elevated serum immunoglobulin levels, their ability to produce specific antibodies may be reduced. For example, HIV-infected children cannot produce antibodies against the specific capsular polysaccharide antigens of Haemophilus influenzae type b and Streptococcus pneumoniae. This deficiency may arise from direct impairment of B lymphocyte function or loss of helper T lymphocytes.

The CD4 molecule as a marker characteristic of helper T lymphocytes can also be found on the membranes of other cell types, including circulating monocytes and macrophages, dendritic cells, NK cells, some B lymphocytes and glia cells. These cells can also be infected by the virus and be destroyed in the process of virus replication or serve as a reservoir for virus latency. Disturbances in the phagocytosis process are a consequence of HIV infection of macrophages and monocytes. In addition, Langerhans cells in the epithelium have been shown to be highly sensitive to HIV infection and may play an important role in the establishment of infection.

**Acquired immunodeficiency syndrome**

AIDS is a set of clinical diseases, primarily opportunistic infections and malignancies, which occur as a result of the destruction of the immune system by HIV. The syndrome is a terminal manifestation of HIV infection that occurred many years earlier (10 years or more). The continuous and inexorable progression of HIV infection is well described and passes through several stages.

**Early acute HIV infection**

Symptoms and clinical manifestations of the disease of early acute HIV infection are frequent and nonspecific. The clinical picture is typical of many viral infections, including those caused by HIV, Ebstein-Barr virus, or cytomegalovirus (CMV). The clinician should consider the possibility of acute HIV infection if the patient has symptoms and signs of viral infection, has risky sexual behavior, or is an intravenous drug user. In 50 to 90% of people, acute illness occurs 2 to 4 weeks after HIV infection. In most cases, the only symptoms are a fever and a mild sore throat. A smaller number of patients may have fever, myalgias, lethargy, pharyngitis, arthralgias, lymphadenopathy and a maculopapular rash on the body. Some patients have aseptic meningitis, and the headache may be due to mild irritation of the soft meninges. The acute illness usually lasts from 3 to 14 days and, as a rule, complete recovery occurs, even in patients with neurological complications. Antibodies against HIV are usually undetectable at first. However, various methods have been developed to detect viral RNA in blood. Although patients with acute HIV infection usually have high levels of viral RNA.

**Diagnosis of HIV infection**

By determining the viral RNA in the blood using the polymerase chain reaction (PCR) method, HIV infection can be detected very early after infection. However, because of its cost, PCR is not used as a screening test, unless the doctor suspects an acute infection. Instead, the screening test for HIV infection is usually the detection of circulating antibodies to the virus by ELISA (Enzyme-linked immunosorbent assay). Unlike most serological tests, in which the presence of antibodies indicates a previous infection, positive anti-HIV antibodies indicate the presence of an active infection. Any presence of anti-HIV antibodies must be considered an active infection that can be transmitted to others. It is important, therefore, that people with positive anti-HIV antibodies are educated about the way the virus is transmitted, in order to minimize the spread of the infection.

Specific anti-HIV antibodies usually appear 6 to 12 weeks after infection. However, PCR tests to detect specific HIV RNA have shown that in rare cases, infected individuals do not develop antibodies for several months or years after exposure to the virus. These persons have false negative serological tests for HIV. In addition, some patients in the terminal stages of AIDS may have negative serological tests (probably due to severe B lymphocyte dysfunction). Fortunately, patients with advanced disease can usually be diagnosed using other specific tests. However, B lymphocyte dysfunction can complicate the serological diagnosis of opportunistic infections that occur in AIDS.

The most common method for testing for the presence of anti-HIV antibodies is the ELISA. This test is performed by adding the patient's serum sample to the previously bound HIV antigens. If antibodies are present, they will form complexes with antigens. Anti-immunoglobulin antibodies linked to the identifying enzyme are then added. Although ELISA is a very sensitive test (> 99%), it is not completely specific, so false-positive results are possible. When screening large populations (eg, all US adults), even a false-positive rate of less than 0.01% would mean that many healthy individuals would be misdiagnosed as HIV positive. Consequently, positive results of the ELISA test must be verified by other specific tests. In most laboratories, such a test is an immunoblot (or "Western blot"), which detects antibodies specific for viral polypeptides. Western blot is a sensitive and specific method for the detection of anti-HIV antibodies, but it is expensive and time-consuming to use for basic screening purposes.

Rarely, some individuals show nonspecific cross-reactivity in HIV serologic tests and are difficult to distinguish from individuals with early HIV infection. The proper diagnostic approach is to repeat the Western blot analysis after 3 or 4 months. By then, a person who is actually infected usually develops new antibodies to different epitopes (proven by Western blot), while those with non-specific reactivity will have the same result as before. Waiting for repeat HIV tests can be very difficult for patients, who need support and education to understand the limitations of modern technology.

Other tests are available to diagnose HIV infection, but they are not always useful. P24 is an antigenic protein of the viral core encoded by the gag gene, and its presence in the blood indicates active viral replication. High levels are detected during acute infection and can be used for diagnosis at this stage. However, in an already established latent infection, this antigen cannot be detected in the serum of all patients and is therefore less useful.

The PCR method for determining HIV RNA is widely used in clinical practice, most often to assess the need and effectiveness of antiretroviral therapy. It is rarely used as a screening test, although it can be useful for diagnosing an acute infection, when specific anti-viral antibodies have not yet appeared. Determination of viral DNA by PCR method is a very sensitive and specific method for early detection of infection. It is most commonly used to identify HIV-infected children whose mothers are HIV-positive, when the presence of maternal antibodies may complicate serological diagnostic tests. Although the DNA test becomes positive somewhat later than the RNA PCR test, RNA can sometimes be undetectable during an established infection, even in the absence of antiretroviral therapy, which is not the case with DNA. Both DNA and RNA PCR tests can give false-positive results in cases of laboratory contamination. Therefore, it is necessary to confirm a positive finding with serological tests. In addition, genetic drift can cause mismatches with the primers used in PCR assays and can give false-negative results. HIV can be cultured from the lymphocytes of most infected persons, but this test is technically difficult to perform and is mostly used only for research purposes.

When should people be tested for HIV infection? People with HIV seek help from doctors at different stages of the disease. Many patients with early HIV infection do not seek medical help and do not know they are infected until they develop AIDS. However, most patients see a doctor before they develop AIDS. Many want to be tested for HIV because they feel some aspect of their lifestyle is risky. Still others seek medical advice with complaints that are not obviously caused by HIV, and are diagnosed by a suspect doctor who advises them to get tested for HIV. Currently, the US Centers for Disease Control and Prevention recommends routine HIV testing of all adults, adolescents, and pregnant women in healthcare settings. The rationale is that infected individuals who do not know they are infected cannot take advantage of advances in life-prolonging therapies or behavioral changes to protect others.

The period after the definitive diagnosis of HIV infection is very difficult for the patient. Anxiety about the possibility of contracting AIDS and worry about passing the virus on to close family members are common reactions. Patient counseling is very important at that point. Patients should learn how the virus spreads and be fully educated about the obligation to protect other people, especially their sexual partners. Patients should be reassured that normal contact with others does not pose a risk for transmission of the virus.

**Consequences of HIV infection**

The probability of disease progression and the occurrence of AIDS can be estimated by determining the degree of immunodeficiency. The most useful is the determination of the level of circulating T lymphocytes. CD4+ helper T lymphocytes are a specific target of HIV infection, so determining the number and percentage of these cells in the circulation indicates the degree of damage to the immune system and the risk of developing AIDS. The normal number of CD4+ T lymphocytes in adults ranges from 800 to 2,000 cells/mm3. Constant loss of these cells over time is a common feature of the progressive course of HIV infection. During the latency period, there is usually a continuous loss of CD4+ T lymphocytes and disruption of lymphatic tissue architecture. Eventually, the number of CD4+ T lymphocytes in the blood also decreases. The probability of the appearance of opportunistic infections increases drastically when the number of CD4+ T lymphocytes decreases below 200 cells/mm3, and the type of infection to which a person is susceptible varies depending on the degree of immunodeficiency. The dynamics of CD4+ T lymphocyte depletion over time may have an important prognostic role. In addition, the amount of virus in the blood, expressed by measuring the level of HIV RNA in the plasma, also provides important prognostic information, perhaps even more important than the CD4+ T lymphocyte count. A higher amount of virus in the plasma is related to a faster progression of the infection to clinically manifest AIDS and death.

Once HIV enters the body, it quickly spreads to many organs, especially the lymphoreticular organs and the brain. The initial infection may be accompanied by a severe, but temporary, loss of CD4+ T lymphocytes (so occasionally opportunistic infections may occur at this stage). With the establishment of the balance of the cellular immune response, the level of viral RNA in the plasma decreases dramatically. However, a high level of active viral replication continues in the lymph nodes. HIV infection is a state of activation of the immune system with a continuous fight between the virus and CD4+ T lymphocytes, which occurs daily until the lymphocyte reserve of the organism is exhausted. The lymph nodes are gradually destroyed, the virus escapes immune surveillance and a high level of the virus appears in the plasma, which is directly correlated with the progression of the disease.

Symptoms or clinical manifestations that suggest on an immunodeficiency state also help identify patients with more rapid progression to AIDS. Therefore, it is necessary to pay special attention to the appearance of fever, night sweats, loss of body weight, or the sudden and unexplained appearance of diarrhea. The presence of an infection caused by Candida in the mouth (sor), also indicates a poor prognosis. Despite these prognostic markers, it is impossible to predict exactly how quickly the disease will progress. The mechanisms that control the rate of progression have not been fully elucidated. Factors such as host genetic predisposition, host immune response, phenotype and viral load are potentially important factors.

Since most people infected with HIV develop progressive immunodeficiency, special attention should be paid to occult infections that can be problematic in the later stages of the disease. Tuberculin skin test and chest X-ray are routine diagnostic tests in HIV-positive patients, because the risk of reactivation of the tuberculosis focus increases dramatically. Patients with a positive skin test, and in the absence of active tuberculosis, should be treated prophylactically with isoniazid. Patients with positive serological tests for syphilis should be treated with anti-treponemal therapy, especially if neurosyphilis is suspected. Basic tests for detection of antibodies against cytomegalovirus (CMV) and Toxoplasma gondii should be performed in HIV-positive patients, as they are at increased risk for reactivation of these infections. HIV-positive individuals are also candidates for pneumococcal vaccination and annual influenza vaccination. Since these patients are at risk for hepatitis B, they should be tested for antibodies against the virus, and those who are negative should be advised to get vaccinated.

**Progression of infection to AIDS**

Acute HIV infection is followed by a latent period during which infected persons often do not have any symptoms of the disease and feel healthy. The duration of the latent period is variable, ranging from a few months to more than 15 years, the average time being about 10 years. Although this period is considered asymptomatic, many patients actually have complaints, which are not obviously related to HIV infection or associated immunodeficiency. For example, people infected with HIV complain of skin changes. The reason for this is unknown, but infection of the epidermal Langerhans cells may be one factor. Many patients have excessively dry or itchy skin, seborrheic dermatitis with eczema, folliculitis or psoriasis. The appearance of severe psoriasis, eczema or folliculitis in previously healthy adults indicates the possibility of HIV infection. Reactivation of herpes zoster can also be a manifestation of HIV infection. Patients are also prone to recurrent infections caused by Herpes simplex virus, Molluscum contagiosum and drug allergies. In addition, a type of skin cancer, cutaneous Kaposi's sarcoma, can be the first manifestation of AIDS. It is characterized by the appearance of blue-purple, palpable, non-pruritic, painless lesions.

Patients in the latent phase of infection may also have localized or generalized lymphadenopathy. Painless generalized lymphadenopathy is a common manifestation of HIV infection, but it has no prognostic significance. On the other hand, localized lymphadenopathy or changes in already enlarged nodes may be early signs of infection or malignancy, and should always be investigated.

Recurrent mucocutaneous candidiasis (vaginal or oral in women, oral in men) and extensive aphthous ulcerations in the mouth are frequent early manifestations of HIV infection. These conditions indicate that the patient has an increased risk of disease progression to AIDS. Hairy leukoplakia (plaques of thickened mucosa on the tongue and other parts of the oral mucosa) is caused by the Ebstein-Barr virus and also occurs early in the course of the disease. Another characteristic of the early stage of the disease is the appearance of abnormal laboratory findings. For example, isolated hematological cytopenias, most often anemia, lymphopenia or thrombocytopenia, occur in many infected persons. Abnormalities in liver function tests are common and are usually the result of previous or current viral hepatitis.

The condition of patients during the progression of HIV infection is routinely assessed in three ways:

• clinical assessment of conditions related to HIV infection or AIDS

• determining the number of CD4+ T lymphocytes

• determining the level of viral RNA in the blood

The decision to initiate antiretroviral therapy is usually based on one or more of the three aforementioned principles of standard assessment. The question of whether to start antiretroviral therapy immediately at the onset of HIV infection remains controversial. In the past, the presence of disease characteristic of AIDS, the number of CD4+ T lymphocytes below 350 cells/mm3 and pregnancy were the criteria for starting therapy. With increasing efficacy, tolerance and availability of antiretroviral drugs, treatment is now initiated at earlier stages of infection.

When antiretroviral therapy was in its infancy, there were only a few drugs available. The use of the first, zidovudine, a nucleoside inhibitor of viral reverse transcriptase, showed that the onset of AIDS could be delayed, but that some indicators of progression, such as depletion of CD4+ T lymphocytes and an increase in viral RNA levels, continued to progress over a period of several months despite applied therapy. Disease progression despite treatment with antiretroviral therapy was the result of the emergence of HIV resistance. In the case of zidovudine and other reverse transcriptase inhibitors, mutations occur in the gene encoding the viral enzyme. Similarly, mutations in the gene encoding the protease are associated with protease inhibitor resistance. The emergence of resistance can be prevented by combining several different antiretroviral drugs that act at different stages of viral replication. Current treatment protocols include two or three drugs (eg. two reverse transcriptase inhibitors and one protease inhibitor). A combination of antiretroviral drugs usually induces a long-lasting reduction in viral RNA levels and an increase in the number of CD4+ T lymphocytes. Such protocols have contributed significantly to the reduction of AIDS deaths in the US and the longer survival of patients who respond to treatment. These protocols are called "highly active antiretroviral therapy" or "HAART."

Pneumonia caused by P. jiroveci is a preventable disease. Prospective studies of HIV-positive patients have shown that at least one third of patients with a CD4+ T lymphocyte count of less than 200 cells/mm3 develop this pneumonia within 3 years, in the absence of prophylactic therapy. The risk is significantly higher if the patient has other symptoms (fever, night sweats, weight loss or oral candidiasis). The risk of infection is greatly reduced by the use of prophylactic therapy, such as trimethoprim/sulfamethoxazole.

**Clinical manifestations of AIDS**

"AIDS-related diseases" are primarily due to increased susceptibility to infections and some malignant tumors as a result of immunodeficiency. Most infections are caused by intracellular pathogens that are controlled by the cellular immune response. These infections are more often the result of endogenous reactivation of the focus of infection than newly acquired infections.

**Diseases associated with AIDS**

* Multiple or recurrent bacterial infections (two in a 2-year period) in children under 13 years of age: septicemia, pneumonia, meningitis, bone or joint infection, or internal abscess caused by H. infuenzae, streptococcus, or other pyogenic bacteria.
* Candidiasis of the esophagus, trachea, bronchi or lungs
* Disseminated coccidioidomycosis
* Extrapulmonary cryptococcosis
* Chronic cryptosporidiosis, with diarrhea lasting more than 1 month
* Cytomegalovirus infection
* Mucocutaneous infection caused by herpes simplex viruses that persists for more than 1 month
* HIV encephalopathy
* Disseminated histoplasmosis
* Isosporiasis, with diarrhea lasting more than 1 month
* Kaposi's sarcoma
* Primary brain lymphoma
* Non-Hodgkin's lymphoma of B lymphocytes or unknown phenotype, including Burkitt's lymphoma
* Lymphoid interstitial pneumonia in children younger than 13 years
* Disseminated mycobacterial infection (not caused by M. tuberculosis)
* Extrapulmonary tuberculosis
* *Pneumocystis jiroveci* infection
* Progressive multifocal leukoencephalopathy
* Recurrent infections caused by salmonella
* Toxoplasmosis of the brain

Each of these diseases indicates the diagnosis of AIDS in the presence of laboratory evidence of the presence of HIV infection.

**Lung infections**

Pneumocystis jiroveci pneumonia (PCP) is the most common opportunistic infection associated with AIDS, occurring in 25 to 60% of patients. Typical symptoms are fever, cough and shortness of breath. Although this pneumonia responds to the applied therapy, it is still associated with a mortality rate of 10 to 20% of cases that develop irreversible respiratory failure.

Application of specific antiretroviral therapy and anti-P. jiroveci therapy significantly improved quality of life and length of survival. Many patients are able to continue a normal life for a long time. In patients who achieve an optimal response to antiretroviral therapy, there is usually a significant reconstitution of the cellular immune response, with a concomitant reduction in the risk of opportunistic infections as long as viral replication is suppressed. If patients cannot adhere to therapy, or cannot tolerate side effects, treatment failure and the development of viral resistance can limit the success of antiretroviral therapy. If suppression of viral replication cannot be achieved, the outcome in patients with advanced disease is progressive deterioration with repeated episodes of infection.

**Gastrointestinal infections**

Gastrointestinal problems are common in AIDS patients. Oral and pharyngeal candidiasis is almost universal in patients with severe immunodeficiency, but is usually curable. A significant number of these patients will develop esophageal candidiasis, accompanied by pain and difficulty swallowing with consequent weight loss. Esophageal infection can also be caused by herpes viruses, herpes simplex and CMV, although they show tropism for other tissues. Herpes simplex causes recurrent skin infections that may be resistant to therapy. CMV typically causes disseminated disease accompanied by viremia. Involvement of the large intestine can result in intense abdominal pain and diarrhea. CMV infection of the eye is manifested by blurred vision and can be the cause of blindness.

Diarrhea is a common problem in patients with advanced AIDS and can be serious and difficult to diagnose and treat. It can be caused by a large number of agents:

* CMV and other viruses
* Intestinal Gram-bacteria such as *Salmonella* and *Shigella* spp. (with accompanying bacteremia)
* *Clostridium difficile* as a cause of nosocomial infections
* infections caused by mycobacteria (especially *Mycobacterium avium*) primarily of the small and large intestine, accompanied by malabsorption and diarrhea
* intestinal parasites such as *Giardia*, *Isospora*, *Cryptosporidium* and *Microsporidium* spp.

Malignancies such as Kaposi's sarcoma or lymphoma of the stomach or colon may be accompanied by gastrointestinal symptoms.

Diagnostics of intestinal disorders in AIDS patients involves the cultivation of stool to examine bacteria and microscopy to identify parasites. If these tests do not give an answer, it is necessary to perform invasive tests.

**Infections caused by mycobacteria and fungi**

Although P. jiroveci is a common cause of pneumonia in AIDS patients, other agents such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and intestinal Gram-negative bacilli must be considered. Tuberculosis is another important cause of pneumonia in AIDS and can disseminate and manifest as lymphadenitis, hepatitis or meningitis.

Although most opportunistic infections occur in the phase of severe immunodeficiency, tuberculosis is an exception and can occur earlier, when the function of the immune system is slightly suppressed (in the "asymptomatic" phase). Consequently, HIV infection should be considered in the case of pulmonary tuberculosis in previously healthy young adults and adolescents. Infected patients can easily spread tuberculosis to household members and other people from the close environment. Thus, the increase in the incidence of tuberculosis in the US, which began in the late 1980s, can be largely attributed to the spread of tuberculosis among HIV-infected persons and persons from their environment. Fortunately, tuberculosis in patients with HIV infection is curable and remains one of the few opportunistic infections that does not require lifelong suppression after treatment.

Disseminated infection caused by M. avium complex (MAC) is also common in AIDS. Although the MAC microorganism can cause pneumonia, it usually results in the development of disseminated disease, particularly in the lymphoreticular organs and the gastrointestinal tract. Patients usually suffer from fever, night sweats, weight loss, splenomegaly, and hepatomegaly, while some develop diarrhea. Similar symptoms are seen in patients with disseminated fungal infections, such as histoplasmosis, which are common in patients in the Midwest and Latin America. Disseminated coccidiomycosis is seen in patients in the southwestern United States.

**Infections of the nervous system**

Opportunistic infections can cause neurological manifestations in AIDS patients. Fever and headache are the most common clinical manifestations of infection caused by Cryptococcus neoformans, which usually causes meningitis. There are other opportunistic infections that can cause changes in the brain. Reactivation of infection with the parasite T. gondii typically causes multifocal brain infection. Patients may experience headache, confusion, or seizures. CMV causes retinitis and occasionally encephalitis.

**Direct manifestations of HIV infection**

By itself, HIV can directly affect changes in many organs, such as the intestines and kidneys. HIV nephropathy is manifested by proteinuria, nephrotic syndrome and renal failure, which responds to antiretroviral therapy. Myopathy and myositis can occur as a consequence of the presence of HIV or as an effect of the applied therapy. Cardiomyopathy also occurs. One of the disturbing features of advanced AIDS is emaciation, characterized by intense loss of body and muscle mass. The pathogenesis of this syndrome is not fully explained, but it indicates a poor prognosis.

**Oncological manifestations of advanced AIDS**

It is important to realize that the types of AIDS-related diseases vary in frequency of occurrence and significance. The treatment and prevention of infections like PCP has greatly improved, so some other infections (eg those caused by mycobacteria and CMV) are taking over. Neurological manifestations of HIV infection in advanced stages of the disease and malignancy are of increasing importance. As patients live longer with severe immunodeficiency, malignancy is seen more frequently. The two most common malignancies associated with HIV are Kaposi's sarcoma and lymphoma. However, as the life of patients with HIV infection is prolonged, as a result of effective antiviral therapy, other tumors are also seen more frequently (eg, lung tumor).

Kaposi's sarcoma is associated with an infection caused by HHV-8. Its appearance is independent of the degree of immunodeficiency, although it is more aggressive and more difficult to treat if the number of CD4+ T lymphocytes is low. In its mildest form, it manifests itself as a localized skin lesion without significant symptoms. Severe cases of Kaposi's sarcoma manifest as widely disseminated lesions, involving the lymph nodes, gastrointestinal tract, and lungs, which can be fatal.

Hodgkin's and non-Hodgkin's lymphoma occur more frequently in HIV-infected patients and are becoming an increasingly serious problem. They often involve the central nervous system and usually have a poor prognosis. The basis of most cases of central nervous system lymphoma is an infection caused by EBV, which indicates the possible role of this virus in the pathogenesis of this tumor.

Malignant transformations associated with human papillomavirus infection represent a significant problem for AIDS patients. Cervical cancer is more common in women with co-infection caused by HPV and HIV.

**Neurological manifestations in advanced AIDS**

Although opportunistic infections and lymphomas can have their manifestations on the central nervous system, by far the most common neurological problems are caused by HIV itself. HIV has neurotropic properties. Infection of the nervous system occurs early in the disease, probably shortly after exposure. In the central nervous system, the virus resides predominantly in macrophages, although the pathogenesis of HIV-induced neuronal damage remains unclear.

Neurological manifestations can occur at any stage of HIV infection. Acute primary infection may be associated with complications such as aseptic meningitis, encephalitis, myelitis or inflammatory neuropathies such as Guillen-Barre syndrome. In the later stages of the disease, patients may have peripheral neuropathies, and motor or sensory neurological deficits. The most common form of neurological disease is HIV-associated encephalopathy, which is manifested by progressive dementia. In its earliest stages, HIV-induced dementia manifests as mental retardation, attention deficit disorder, and forgetfulness, and must be distinguished from the depression that often accompanies HIV infections. In the later stages of HIV dementia, patients have severe attention deficits, motor and sensory dysfunctions and may be completely unable to care for themselves. Computed tomography of the brain often shows significant brain atrophy. Severe dementia is inevitably fatal.

**Disease outcome**

Although HIV infection is still incurable and fatal, its progression varies. With improvements in antiretroviral therapy and the use of prophylactic antimicrobial therapy, HIV infection is increasingly becoming a chronic disease. Modern antiretroviral therapy is effective and generally well tolerated.

**Genetic and developmental predispositions for the onset of AIDS**

As mentioned earlier, recurrent vaginal candidiasis can be a common problem in HIV-positive women. In addition, there is increasing evidence that HPV infections are more aggressive in people with HIV infection. Consequently, cervical cancer has become an important complication. Host-specific genetic determinants that may influence the rate of disease progression in HIV infection remain to be elucidated. In addition, there may be patient differences in the toxicity of antiretroviral drugs, depending on genetic predisposition. It should be noted that there are no indications that treatment is ineffective or harmful, so all patients should be treated, regardless of race or gender.

HIV infection in children has a similar course with progressive immunodeficiency, recurrent opportunistic infections and neurological manifestations. However, disease progression can be much faster in infants. Transmission is usually vertical (from mother to fetus), and 13 to 40% of babies born to HIV-positive mothers are infected. Combination antiretroviral therapy during the last two trimesters of pregnancy and during delivery can reduce transmission rates to less than 2%.

Most children with vertically acquired HIV infection have a slow progression of infection (8 years or more) to clinically manifest disease. However, 15 to 20% have an accelerated course of the disease that ends in death within 2 to 4 years after birth, without medical intervention. In addition to opportunistic infections, infants have stunted growth and development, which is a common consequence of HIV infection of the brain. Recurrent bacterial infections (eg, otitis, pneumonia) are common and have underlying V lymphocyte dysfunction. However, as in adults, treatment and prophylactic therapy improve quality of life and length of survival.

**Treatment of HIV positive patients**

Treatment of a patient infected with HIV implies a long-term commitment of the doctor and other members of the health care team not only for the implementation of medical treatment, but also for the support and education of the patient and family. Patients must be informed of symptoms that require prompt medical attention, such as the onset of fever and cough or headache. Physicians must be prepared to face clinical manifestations and recognize important complications. Patients must be educated about modes of transmission to prevent the spread of infection. In addition, members of the health care team should help the patient cope with the altered social dimensions of the disease. Family and friends also need support and education.

Antiretroviral therapy has changed HIV infection from a fatal to a chronic disease. The use of antiretroviral therapy in the early stages of the disease delays the progression of HIV infection. Even if applied at a later stage of the disease, therapy can correct immunodeficiency and significantly prolong life. A number of effective drugs have been developed that act at different points in the viral life cycle: binding to CCR5 (coreceptor), fusion of the viral envelope, retrovirus-specific DNA polymerase, integration into the host genome, and viral protease (Figure 6). Similar to the treatment of tuberculosis, the treatment of HIV infection involves a combination of drugs that act in different phases of virus replication in order to achieve synergism and delay the appearance of resistance (highly active antiretroviral therapy, HAART therapy). As with tuberculosis, non-adherence to the therapeutic regimen often becomes the cause of drug resistance.

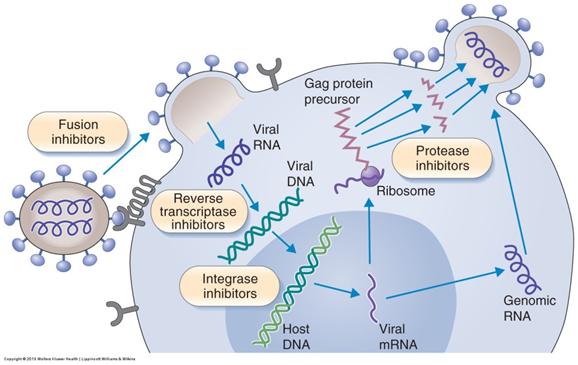


Figure 6.

**Prevention**

For now, the best approach to controlling AIDS is to prevent HIV transmission. Education of the public, as well as of HIV-infected persons, can influence changes in risk behavior and limit the spread of the virus. This includes routine and regular screening of people at risk of HIV infection. HAART therapy has reduced the rate of HIV transmission, indicating that early identification and treatment of those infected with HIV is important for reducing the spread of infection. The development of an effective vaccine would eliminate the threat of this disease in the future, but it is faced with numerous problems. One is the incomplete understanding of the host's immune response to the virus and, although many individuals develop neutralizing antibodies to HIV, their role is not entirely clear. Circulating antibodies effectively remove the virus from the circulation, but do not prevent the progression of the infection. In addition, HIV is subject to antigenic variation. Finally, testing the protective effects of an HIV vaccine in at-risk populations raises ethical questions. Even after an effective vaccine is developed and implemented, AIDS will continue to be an important cause of morbidity and mortality for several decades. Until a vaccine is developed, education and behavioral change, early detection of infected individuals, and antiretroviral therapy remain the most effective ways to stop the spread of HIV.