Teaching unit 15

ARBOVIRUSES AND VIRUSES OF HAEMORRHAGIC FEVERS
PATHOGENIC HUMAN RETROVIRUSES
VACCINES

Arboviruses and hemorrhagic fever viruses

- More than 400 different viruses cause zoonoses worldwide. Reservoirs of these viruses are insects and lower vertebrates
- Viruses that cause zoonoses belong to the RNA family of viruses
- **(*Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae, Arenaviridae , Filoviridae*)**

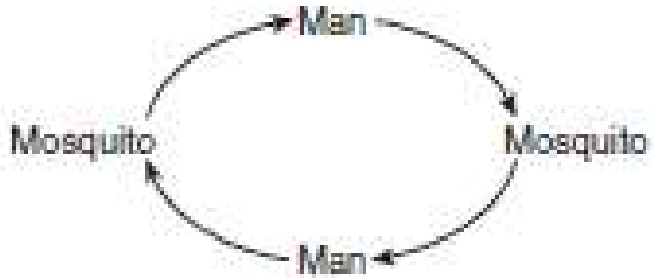
In most cases, zoonotic viruses are named after the place where they were first isolated (for example, St. Louis encephalitis virus) or after the disease they cause (yellow fever virus).

They are divided into two groups:

- **viruses that are transmitted by arthropods**, blood-sucking arthropods (mosquitoes, ticks, flies of the genus Phlebotomus) - **arboviruses**
- **viruses that are not transmitted by arthropods**(transmitted by respiratory route (inhalation of excreta of infected animals), through conjunctiva or direct contact with infected animals)

Arboviruses
-epidemiology-

- Urban dengue and urban yellow fever are easily transmitted between humans and mosquitoes



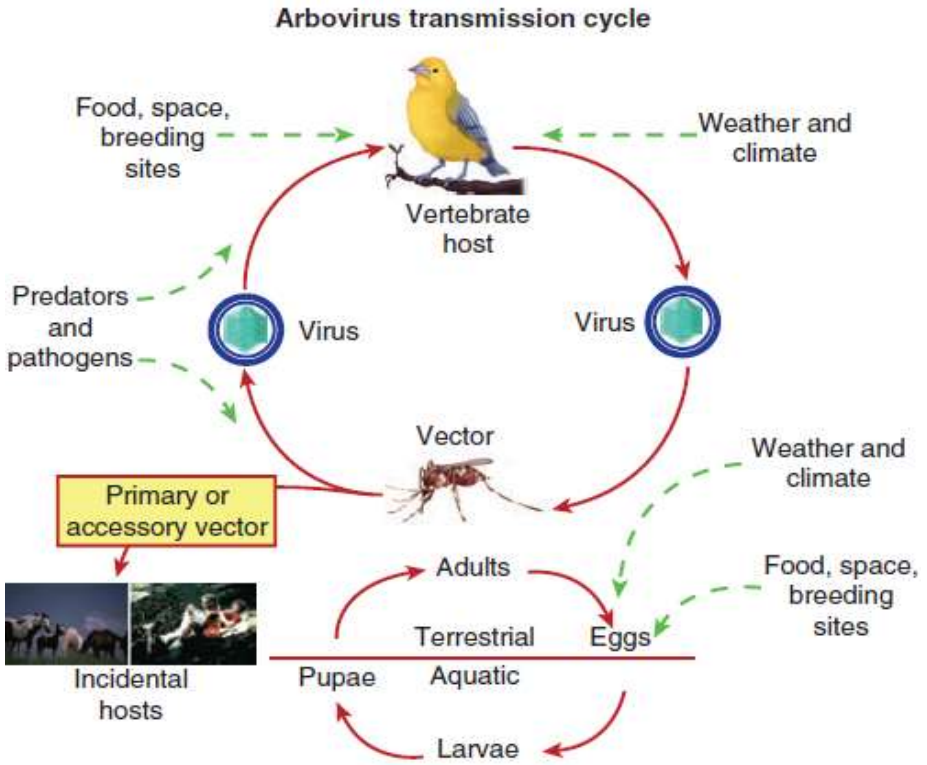
- The life cycle of other arboviruses also includes other vertebrates, mainly small mammals, birds and monkeys

- The infection is transmitted by infected arthropods (by the bite of a mosquito or fly).
- Arthropod infection can be transmitted from generation to generation by transovarial transmission (infection of the arthropods themselves is usually latent and does not cause any damage)

Arboviruses
-epidemiology-

Infected vertebrates are the source of new spread of the virus

The consequences of transmission of infection from arthropods to susceptible vertebrates are variable



- Transient viremia is a feature of infections in which hosts are not the reservoir of infection (mainly humans and higher vertebrates, horses and cats).
- If viremia is maintained for a long period, weeks and months (in some lower vertebrates), then such a vertebrate is a very important reservoir for continuous transmission.

Arboviruses
-pathogenesis-

- After the sting of infected arthropods, viremia and virus replication occur in endothelial cells and mononuclear cells.

- Arboviruses cause three typical clinical manifestations:

1. **Aseptic meningitis or meningoencephalitis** (meningeal and perivascular mononuclear infiltrate, degeneration of neurons and sometimes destruction of supporting structures of neurons)

2. **Liver damage** (necrosis of hepatocytes, degenerative changes in renal tubules and myocardium, microscopic hemorrhages in the brain, lack of coagulation factors - bleeding)

3. **Hemorrhagic fever**

Arboviruses
-pathogenesis of hemorrhagic fevers-

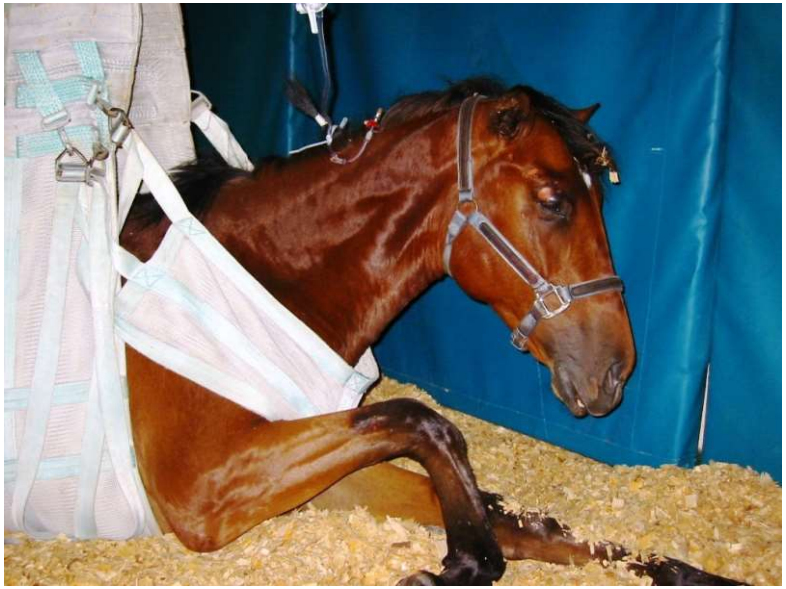
Hemorrhagic fever has been most studied in the case of dengue

- In uncomplicated dengue fever, changes occur in the small blood vessels of the skin (endothelial cell swelling, perivascular edema and mononuclear infiltrate).
- More severe dengue is complicated by shock characterized by perivascular edema and effusions into cavities such as the pleura and hemorrhages. Lymphatic tissue hyperplasia occurs in the spleen and lymph nodes, while areas of focal necrosis are observed in the liver.
- The pathophysiology is associated with **increased permeability of blood vessels and disseminated intravascular coagulation**, which is later complicated by liver and bone marrow dysfunction, thus reducing the production of platelets and the production of coagulation factors in the liver.
- The main abnormalities in blood vessels are mainly provoked by circulating **virus-antibody complexes that activate complement**, followed by the release of vasoactive amines.

Specific arboviruses

Western equine encephalitis virus

- It belongs to the genus *Alphavirus* family *Togaviridae*
- It is transmitted by a mosquito bite ***Culex tarsalis***
- Infection in humans is mostly endemic
- Horses and humans are the final hosts susceptible to infection - clinically manifest disease occurs in 1:1000 infected
- However, in younger people the disease can be very severe
- The disease can vary from a **mild, non-specific and afebrile condition** to **septic meningitis and severe encephalitis** (mortality rate is 5%)



Eastern equine encephalitis virus

- It belongs to the genus *Alphavirus* family *Togaviridae*
- It is transmitted by a mosquito bite ***Culiseta melanura***
- Transmission from mosquito to horses and birds (occasional outbreaks in humans)
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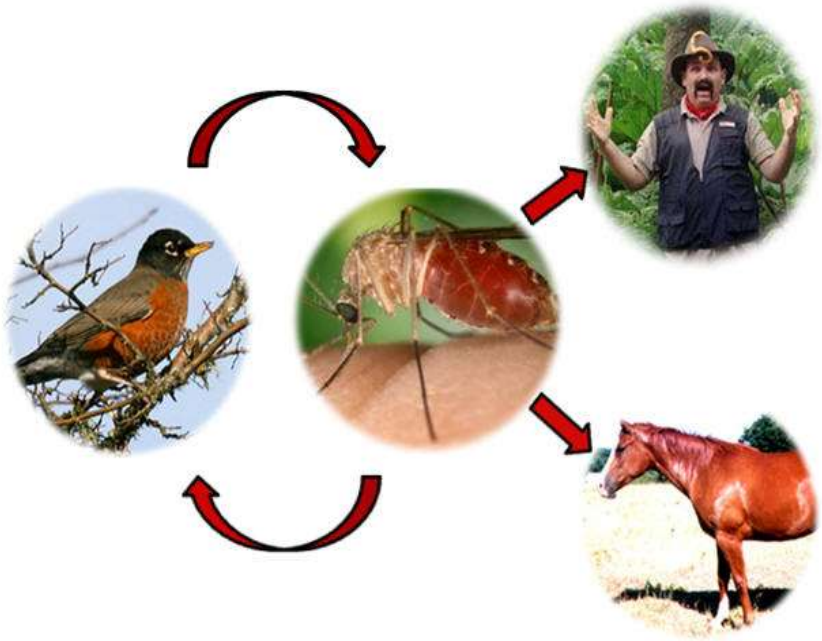
- Causes **severe encephalitis** in horses and wild birds
- The mortality rate in humans is from 33 to 55% regardless of age, and severe sequelae are very common.



St. Louis encephalitis virus

- It belongs to the family *Flaviviridae*
- It is transmitted by a mosquito bite ***Culex tarsalis***
- It infects horses, but does not cause disease in them
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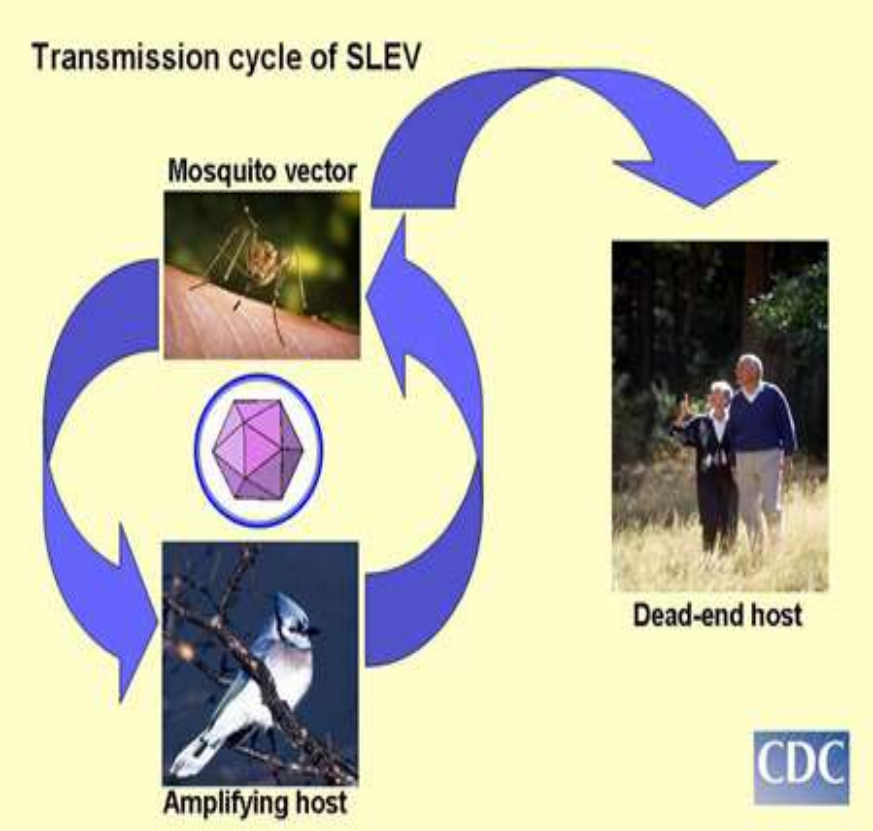
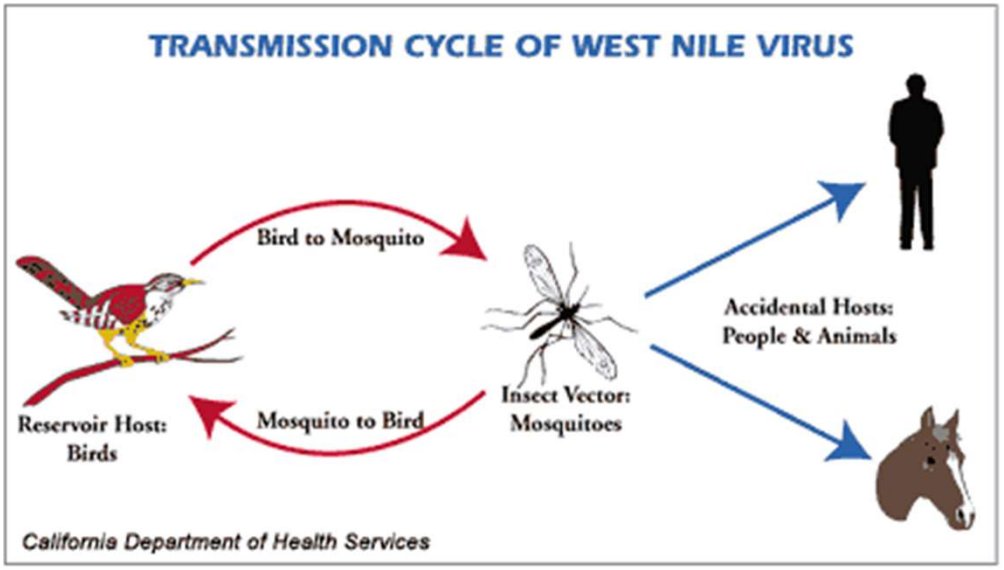
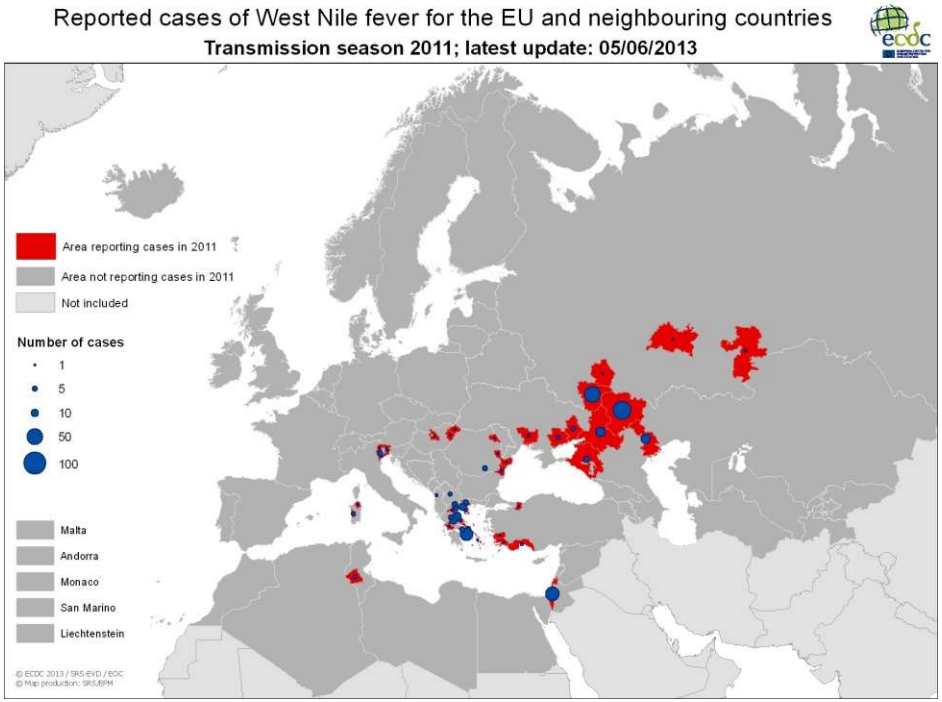
- The spectrum of disease in humans is similar to that of western equine encephalitis
- The highest morbidity and mortality occurs around the age of 40



West Nile virus

- It belongs to the family *Flaviviridae*
- It is transmitted by mosquito bites and the reservoirs are birds
- It can also be transmitted by transfusion, transplant and from mother to child
- After a mosquito bite, the virus replicates in the Langerhans cells of the skin
- The incubation period lasts 3-14 days, on average 3-7 days, followed by viremia and spread of the virus to peripheral organs, sometimes the CNS



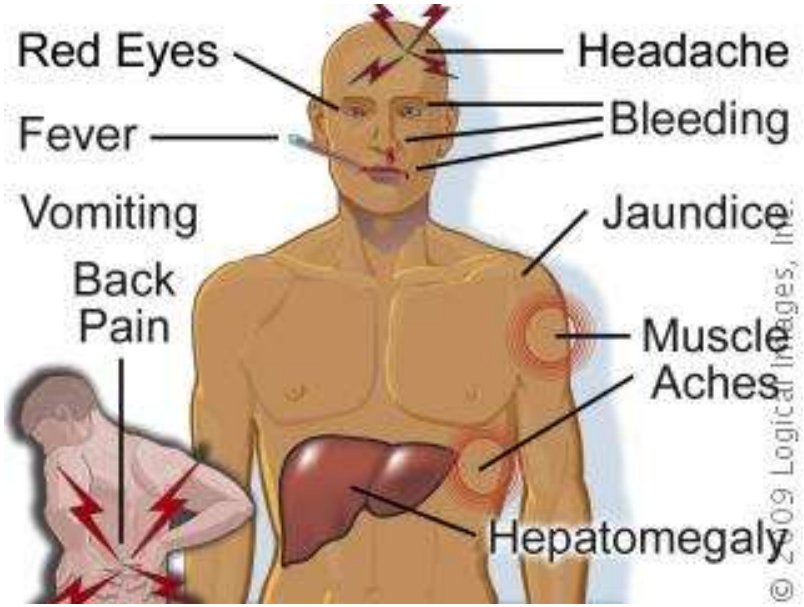


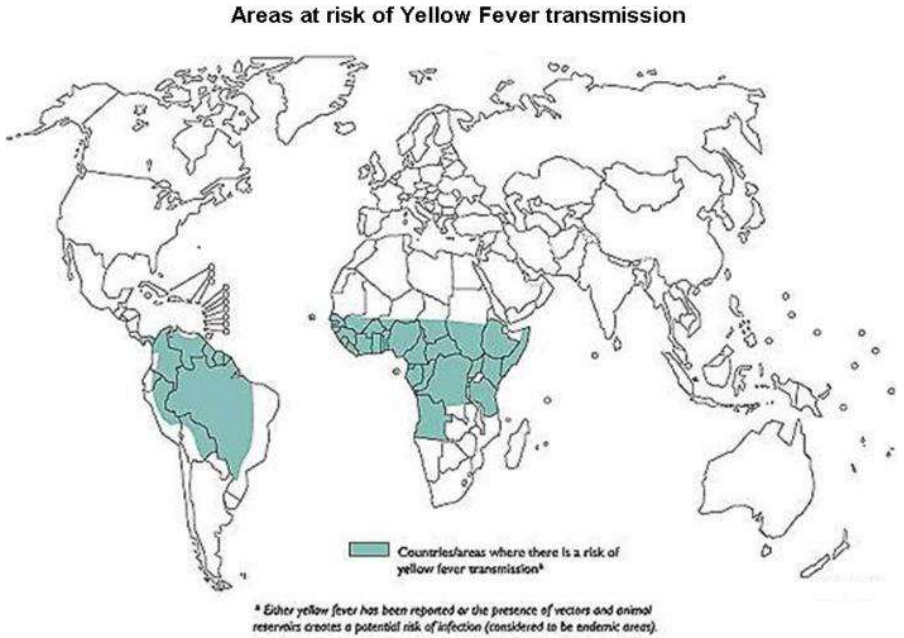
West Nile virus
-clinical manifestations-

1) **asymptomatic infection** (80% of infected people have no symptoms)
2) ***West Nile* fever** (fever, headache, muscle pain; skin rash in half of the cases, mainly on the chest, back, upper extremities; generalized lymphadenopathy; pharyngitis and gastrointestinal symptoms (nausea, vomiting, abdominal pain); the illness lasts 3 to 6 days; children generally have a milder disease than adults
3) **Severe *West Nile* disease** (occurs in 1/150 infected people; the virus in these cases enters the CNS and causes aseptic meningitis, meningoencephalitis, encephalitis or West Nile poliomyelitis, especially in the elderly, which in some cases can end in death; symptoms include headache, fever, stiff neck, disorientation, coma, tremors, convulsions, muscle weakness, paralysis; lasts for weeks and can cause permanent damage and in some cases death; occurs in people older than 50 years and immunodeficient

Yellow fever

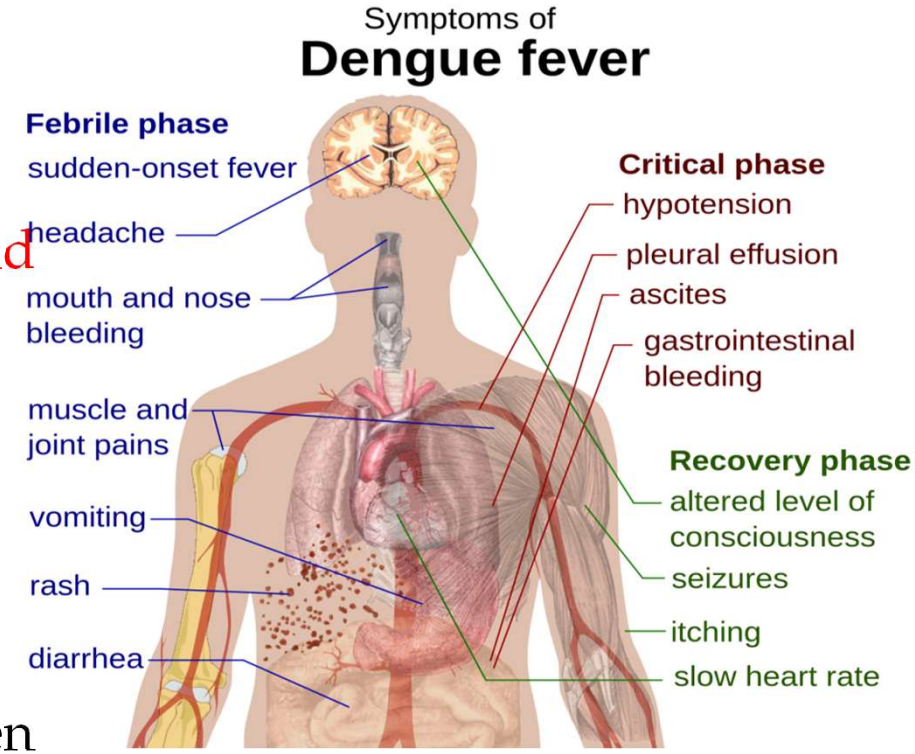
- It belongs to the family *Flaviviridae*
- It is transmitted by a mosquito bite ***Aedes aegypti***
- Clinically, the disease is characterized by the sudden onset of fever, chills, headache, and hemorrhage, which may progress to vomiting with gastric hemorrhage, bradycardia, jaundice, and shock.
- If the patient recovers from the acute episode, there are no long-term sequelae





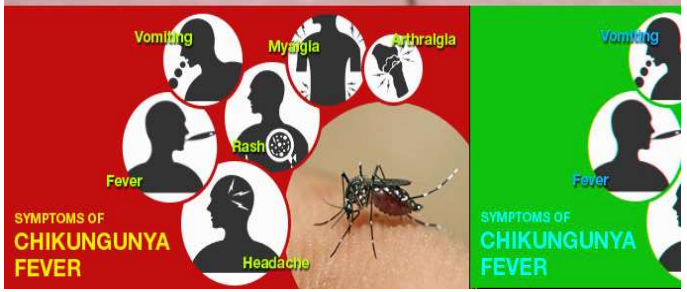
Dengue

- It belongs to the family *Flaviviridae,* there are 4 serotypes
- It is transmitted by a mosquito bite ***Aedes aegypti***
- The known cycle of transmission involves human-mosquito-human transmission, although forest cycles involving monkeys are also possible.
- Incubation lasts 4-10 days, and symptoms last 2-7 days
- The characteristics of the disease are: fever, rash, pain in the back, head, eyes and behind the eyes, muscles, joints, bones, sometimes mild bleeding in the nose, gums, petechiae
- Especially in the Far East, dengue can be characterized by the appearance of **shock, pleural effusions, abdominal pain, vomiting and hemorrhage that can end in death**. The disease is more severe in children



Chikungunya virus

- It belongs to the genus *Alphavirus* family *Togaviridae*
- It is transmitted by a mosquito bite ***Aedes aegypti***
- It is widespread in Asia, Africa, Southern Europe, and the Caribbean islands
- It persists in the forest cycle in primates as reservoirs
- The incubation period is between 2-12 days
- The disease is characterized by a sudden onset of fever followed by **severe myalgia and polyarthritis**
- Symptoms usually last for a week, but musculoskeletal disorders can persist for months
- The disease is usually not fatal and there is no specific treatment or vaccine

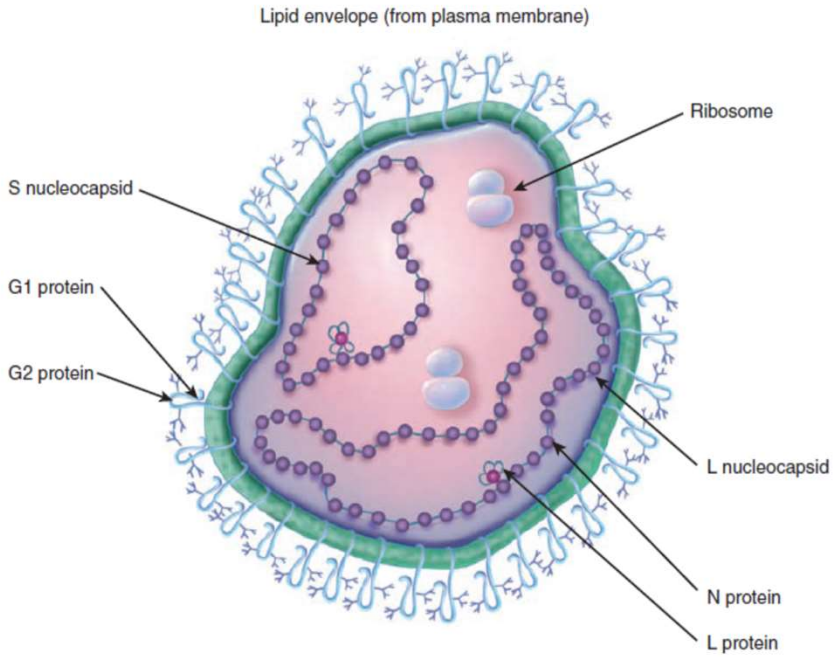


Viruses that cause zoonoses and are not transmitted by vectors

Arenaviridae
Filoviridae
Bunyaviridae (Hantavirus)

Arenaviridae

- They have an envelope
- They are bisegmented, containing a large single-stranded (L), negative and small (S) (−/+) RNA chain
- They have 2 separate nucleocapsids L and S in which RNA segments are packaged
- The envelope contains two viral glycoproteins, G1 (interacts with the cell surface receptor (αDG)) and G2
- The virion also contains host ribosomes inside which give it a granular appearance

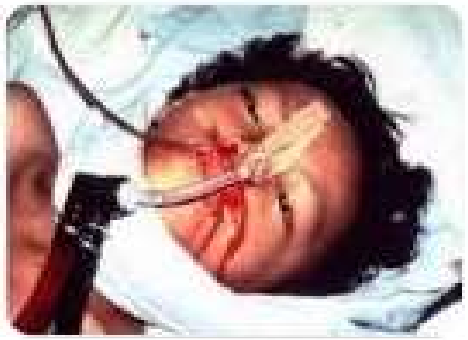


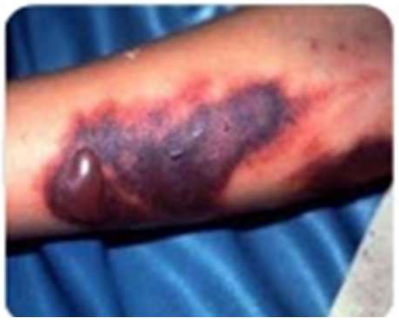
- ***Lassa* virus** in West Africa
- ***Junin, Machupo, Guanarito* and *Sabia* virus in South America**

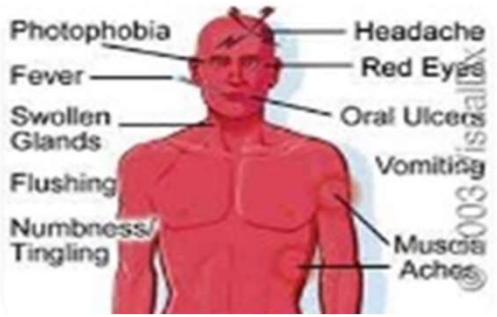
- **They are transmitted by the secretions of infected rodents**
- **They cause hemorrhagic fevers in humans**

Arenaviridae
-hemorrhagic fevers-

- They are transmitted from infected rodents to humans, but it is rarely possible to spread from human to human through contact with secretions and body fluids.
- There are also cases of imported viruses in non-endemic areas (high risk of infection spread)
- Clinically, they are characterized by fever associated with hemorrhages, shock, neurological disorders, bradycardia
- ***Lassa* fever** is mainly accompanied by hepatitis, myocarditis, pharyngitis and acute onset of deafness (mortality rate - 10-50%)

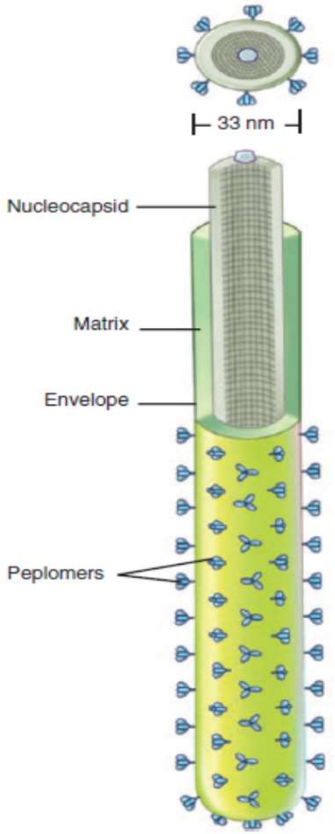


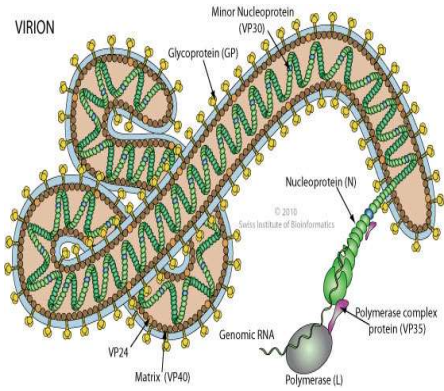


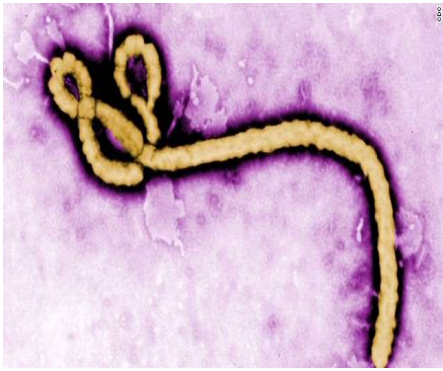


Filoviridae

- Hemorrhagic fevers are caused by two viruses: **Marburg** (1967 epidemic in Yugoslavia) and **Ebola** (1975 epidemic in North Africa)
- They have an envelope, single-stranded negative RNA and a nucleoprotein with helical symmetry (filomorphic appearance of the virion).
- They cause **zoonoses** (the reservoir is mostly small mammals, mostly rodents)
- Large lilies are natural hosts for the Ebola virus
- When the virus is transmitted from animals to humans, then **interhuman** transmission is the more likely mode of spread of infection, mainly through **close contact with bodily fluids of infected individuals**.







Marburg and Ebola
- clinical manifestations -

- The incubation period is 2-21 days, on average 10 days
- **The first manifestations are flu-like headache**, fever, muscle and joint pain, sore throat, diarrhea, vomiting and stomach pain.
- Red maculopapular measles, hiccups, and **internal and external bleeding** may occur
- People who develop severe disease have **hemorrhaging** in the digestive tract and elsewhere
- **Shock and insufficiency** of numerous organs develop
- Mortality extremely high

Marburg and *Ebola* -pathogenesis-

- **Marburg virus replicates in vascular endothelial cells and causes necrosis of these cells. The Ebola virus replicates very quickly and disrupts cellular protein synthesis and the immune response**

- **Both specific and non-specific immunity are suppressed mainly due to infection of monocytes and macrophages**

- Secretory GP of Ebola virus interacts with neutrophils, inhibits their early activation and allows the virus to infect monocytes and macrophages and cause cellular damage

- The release of cytokines is enormous, and the consequence is inflammation and fever

- In addition, viral entry into endothelial cells causes damage to the vascular endothelium, which contributes to hemorrhagic fever

Bunyaviridae (Hantavirus)

- The only one *Bunyavirus* which causes zoonoses and is not transmitted by arthropods
- It causes two clinical forms:
  1) ***Hantavirus* hemorrhagic fever**
  2) ***Hantavirus* pulmonary syndrome**

- The reservoir of infection is rodents
- The virus is transmitted to humans mainly by inhalation of infected rodent secretions, through the conjunctiva or by direct contact with damaged skin

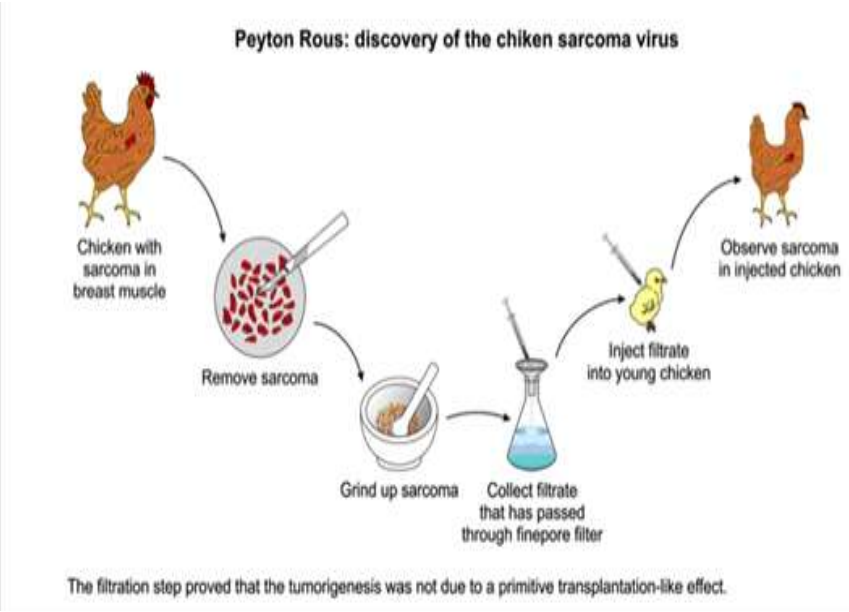
HUMAN RETROVIRUSES

HTLV-1 HTLV-2

HIV-1 HIV-2

History of retrovirology

- The complex relationship between viruses and tumors - the role of viruses in the development of tumors
- The first retrovirus discovered in 1911 - RSV (Rous sarcoma virus) - the cause of tumors in chickens that can be transmitted via tumor extract
- Peyton Rous – Nobel Prize in 1966



History of retrovirology

Reverse transcriptase

Contrary to the basic principles of molecular biology

Genetic information

written in the RNA molecule

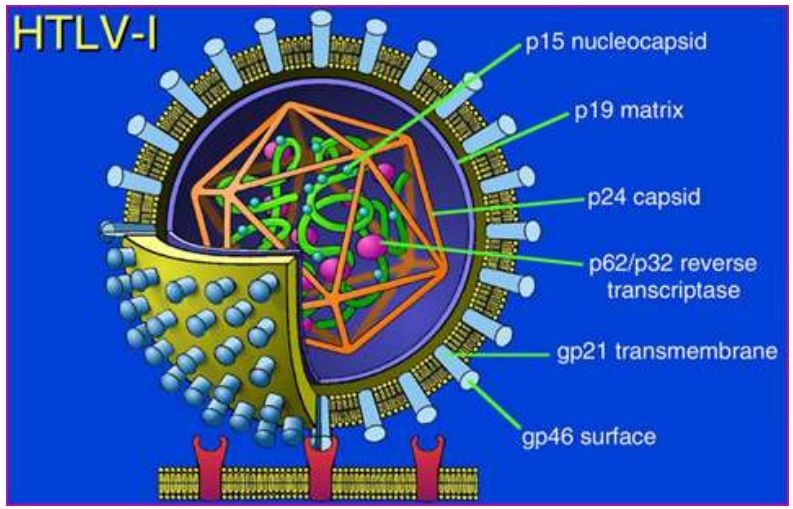
↓

Synthesis of complementary DNA that eventually integrates into the genome of the host cell (provirus), a transitional form in the replicative cycle of the virus

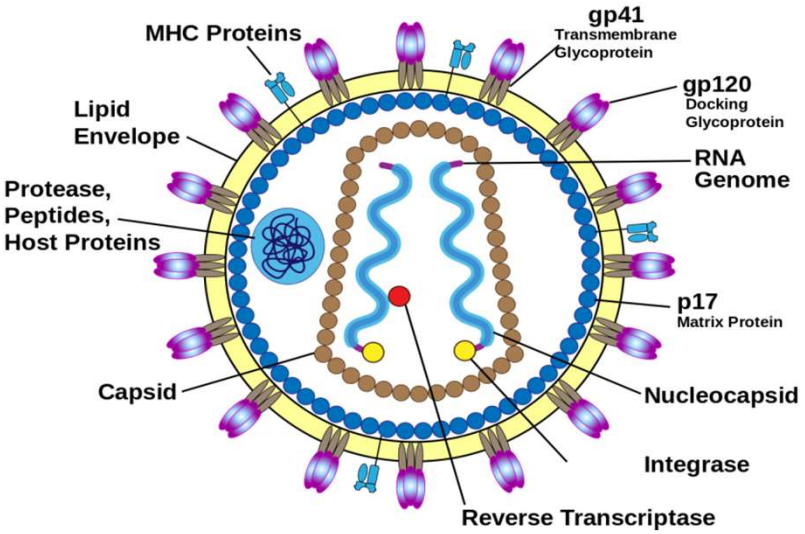
History of retrovirology

- Early 1960s - feline leukemia virus
- 1986 - feline immunodeficiency virus - similar to HIV
- Since 1980 - two groups of retroviruses capable of causing disease in humans have been isolated and described

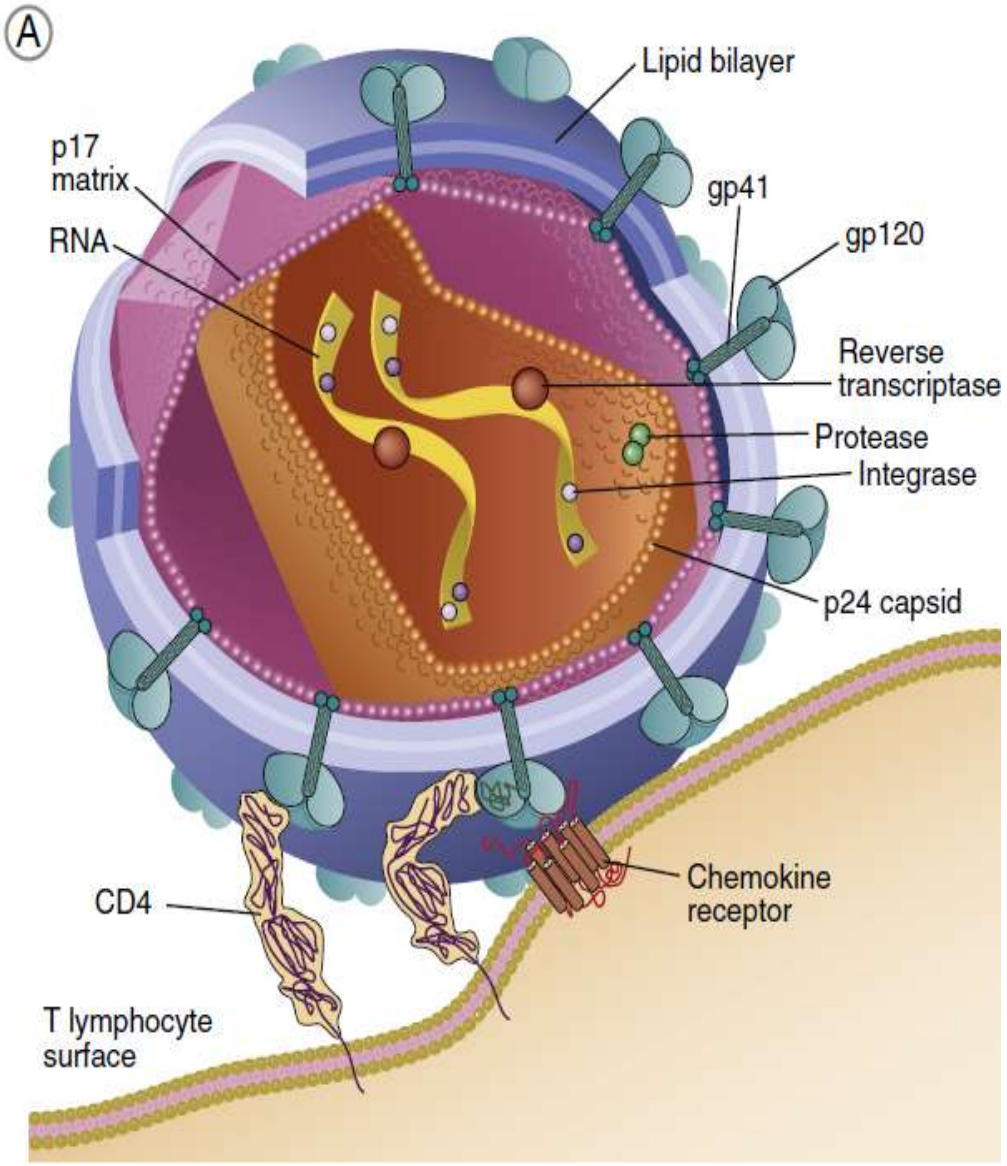
HTLV-1 and HTLV-2



HIV-1 and HIV-2

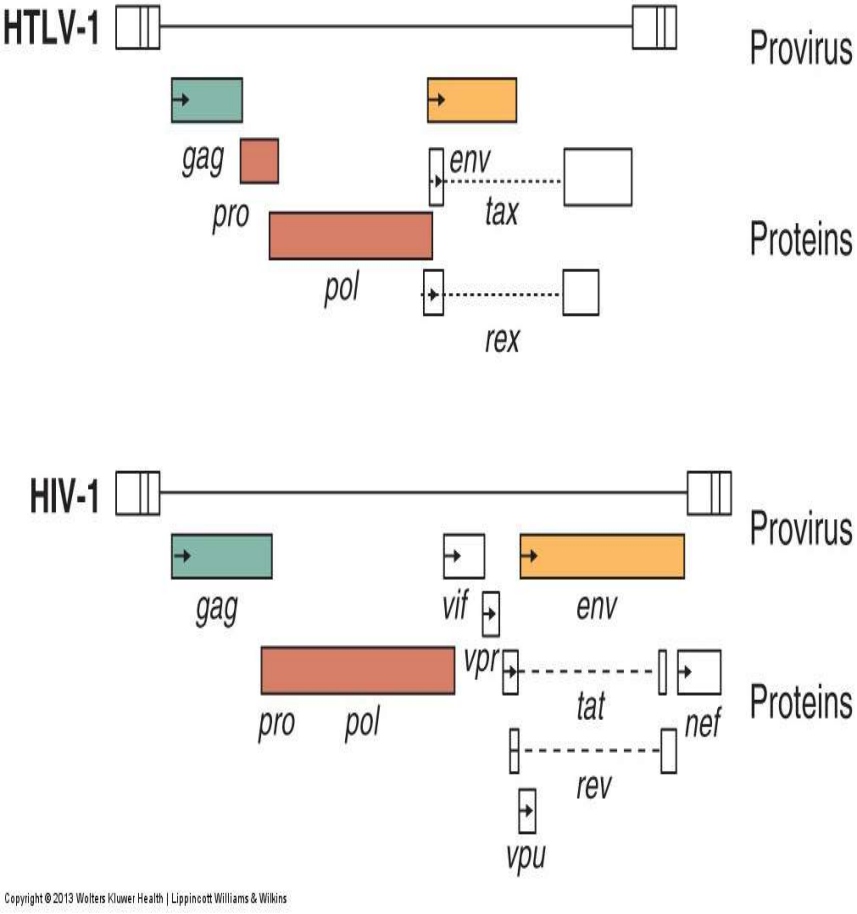


HIV
Structure of the virion



- A small, spherical virus surrounded by a lipid envelope
- Glycoproteins of the viral envelope : **gp120** and **gp41**
- An icosahedral capsid : **p24** and matrix protein **p17**
- The genome contains two identical RNA molecules
- The enzymes **reverse transcriptase, integrase and protease** are attached to the genome

HIV
Structure of genom



Four viral genes are essential for retrovirus replication:

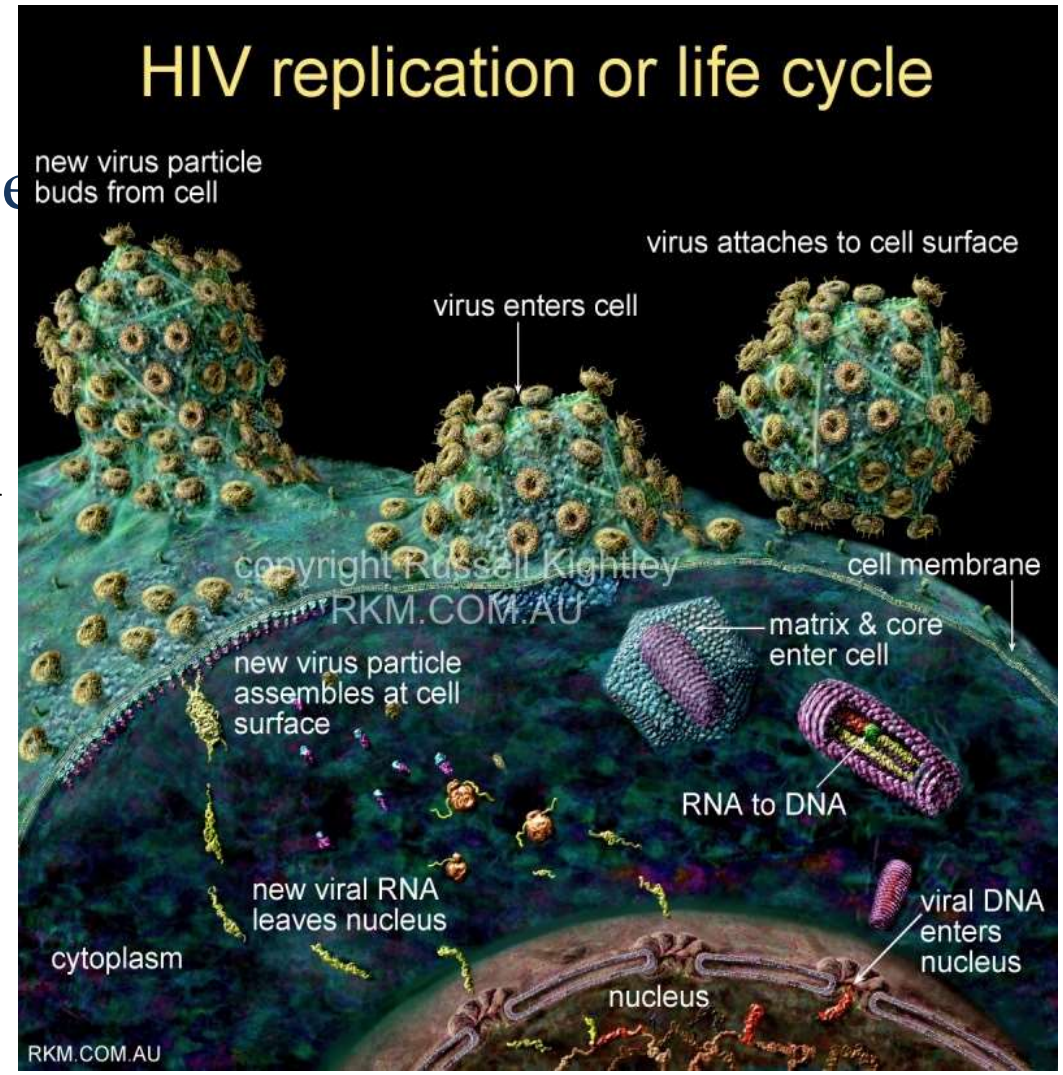
- ***Gag* gene** encodes several core (Gag) proteins of the viral envelope
- ***Pol* gene** encodes **reverse transcriptase** or RNA-dependent DNA polymerase (Pol), an enzyme responsible for genome replication, as well as **integrase**, an enzyme required for the integration of viral DNA into the host cell genome
- ***Env* gene** encodes two viral envelope glycoproteins **gp120** and **gp41**
- ***Pro* gene** encodes a **protease** necessary to cleave Gag and Pol proteins and create their active form

HIV contains at least six other genes that encode proteins that are important in the regulation of complex viral replication

HIV
-entry into the host organism-

- **Via infected cells,** such as macrophages, lymphocytes or spermatozoids or as a free **viral particle**

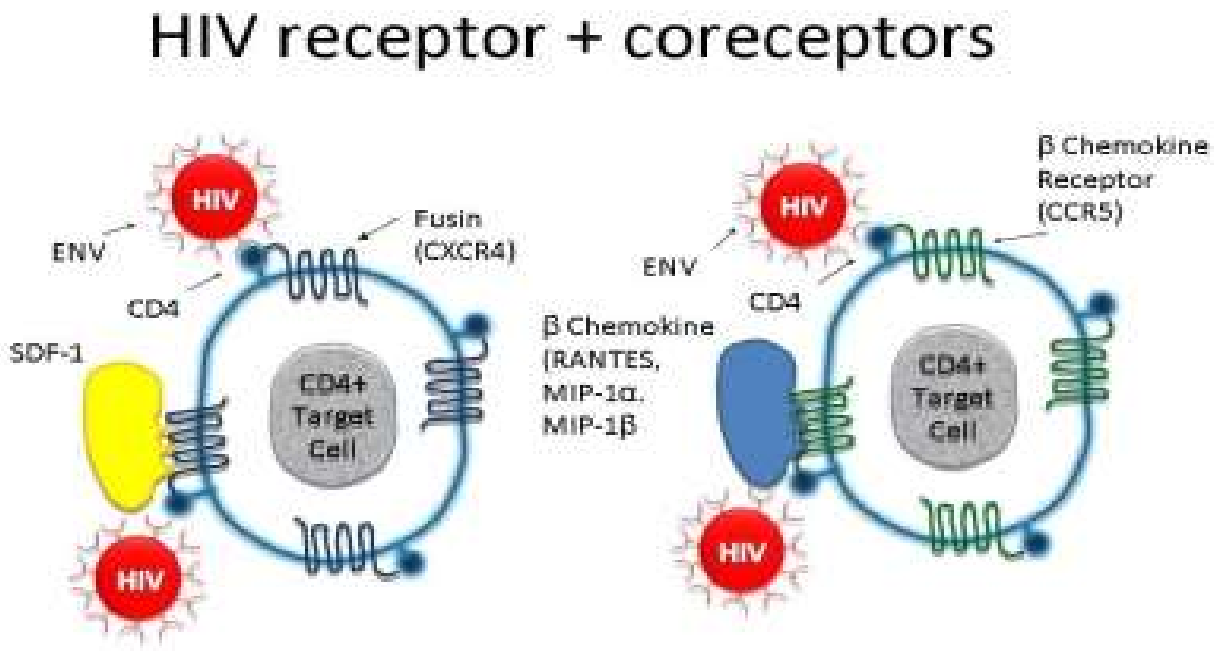
- Through microabrasions on the surface of the mucous membrane, penetration through intact skin after a needle puncture or through undamaged mucosal surfaces



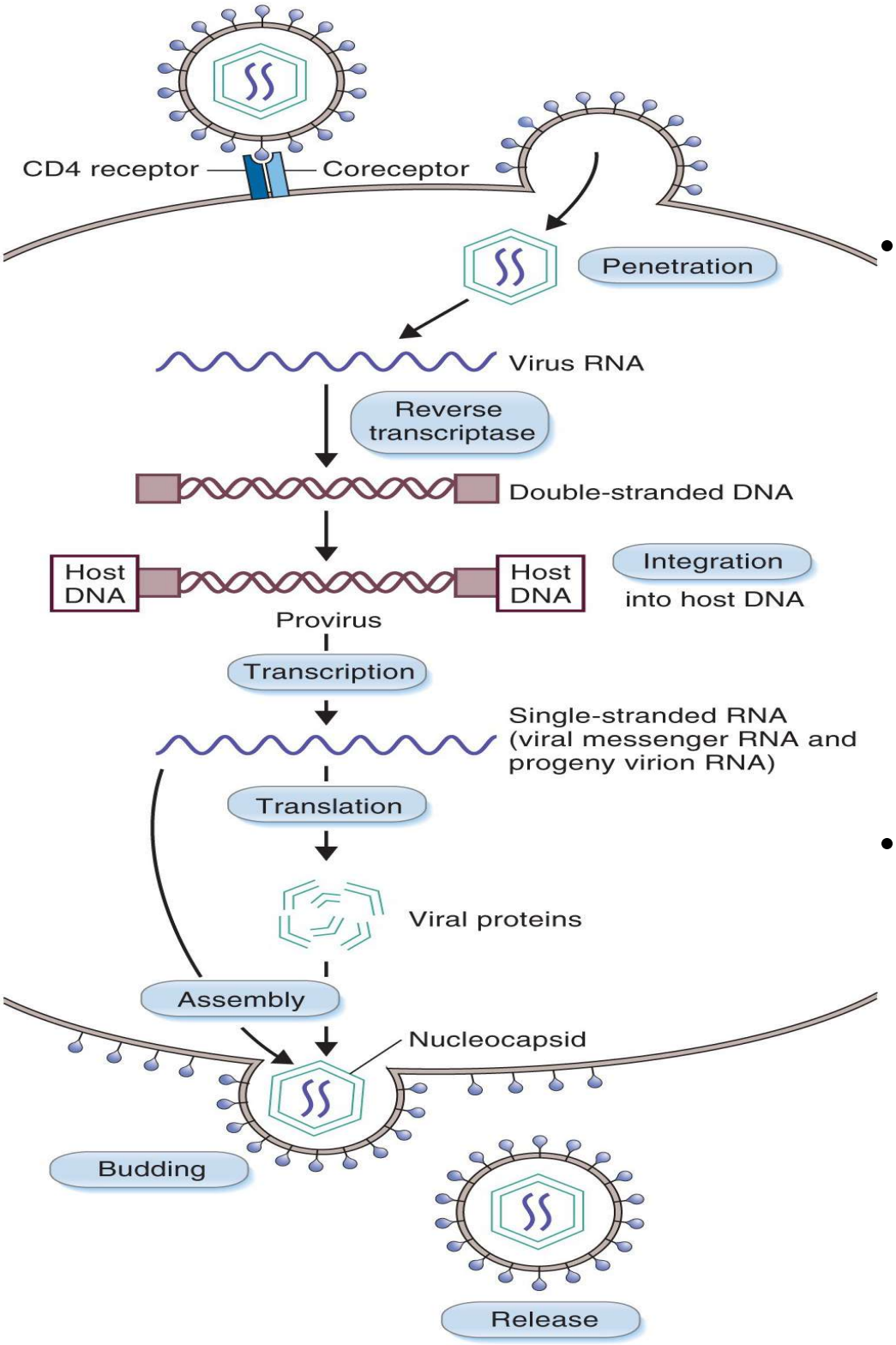
HIV
-spread in the host organism-

- Although HIV can infect many types of cells, two main groups of cells in the body serve as targets for 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).

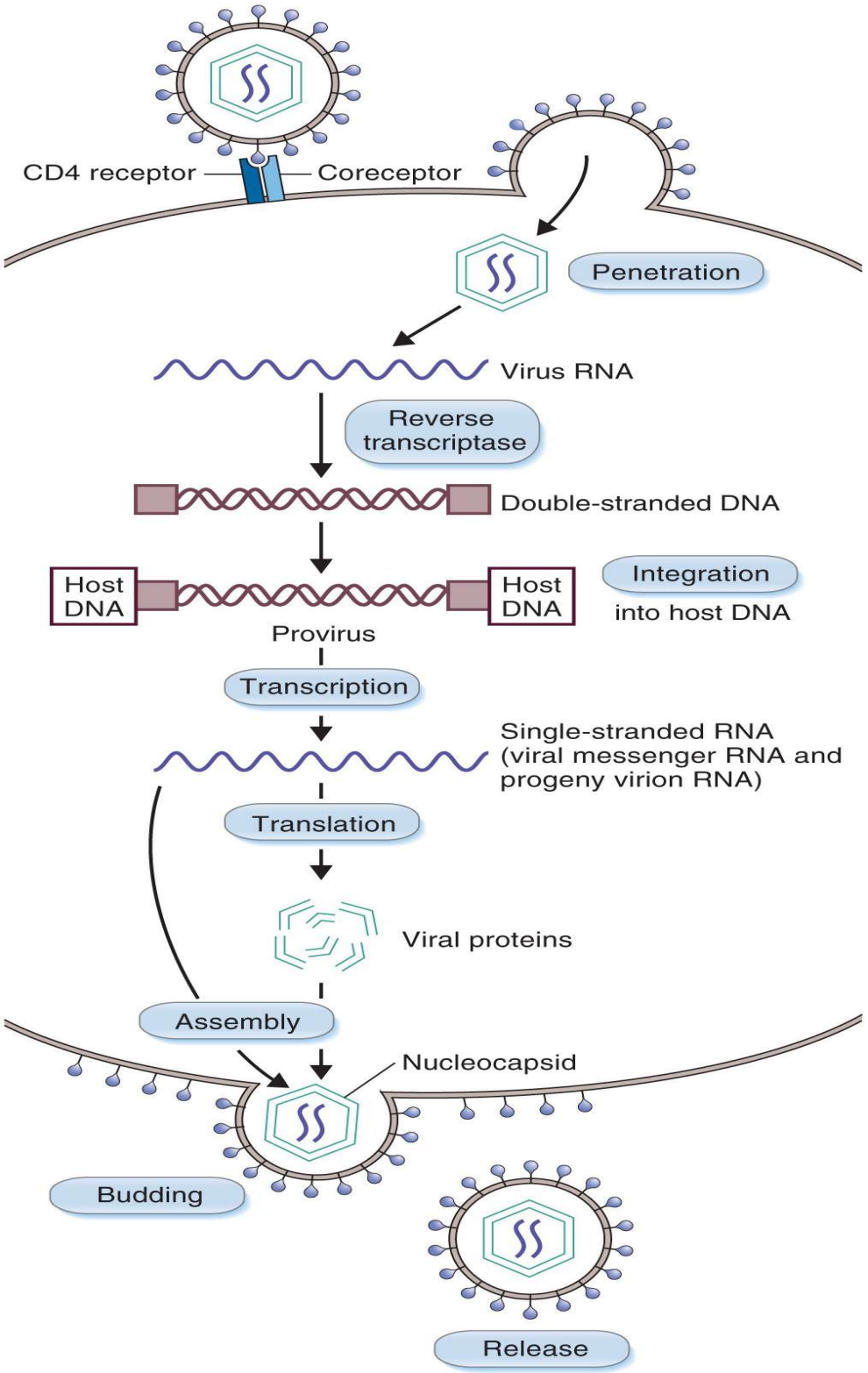


HIV
-replication-



- Binding (adsorption): HIV binds to the **CD4** molecule via the envelope glycoprotein **gp120**. After binding to the CD4 molecule, gp120 binds to one of the two co-receptor molecules (**CCR5** or **CXCR4**), which allows the virus to bind tightly to the cell membrane and the conformational changes of the gp41 protein bringing its hydrophobic domain into contact with the cell membrane
- **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. The viral core, which contains genomic RNA and reverse transcriptase molecules, is released into the cytoplasm

HIV
-replication-

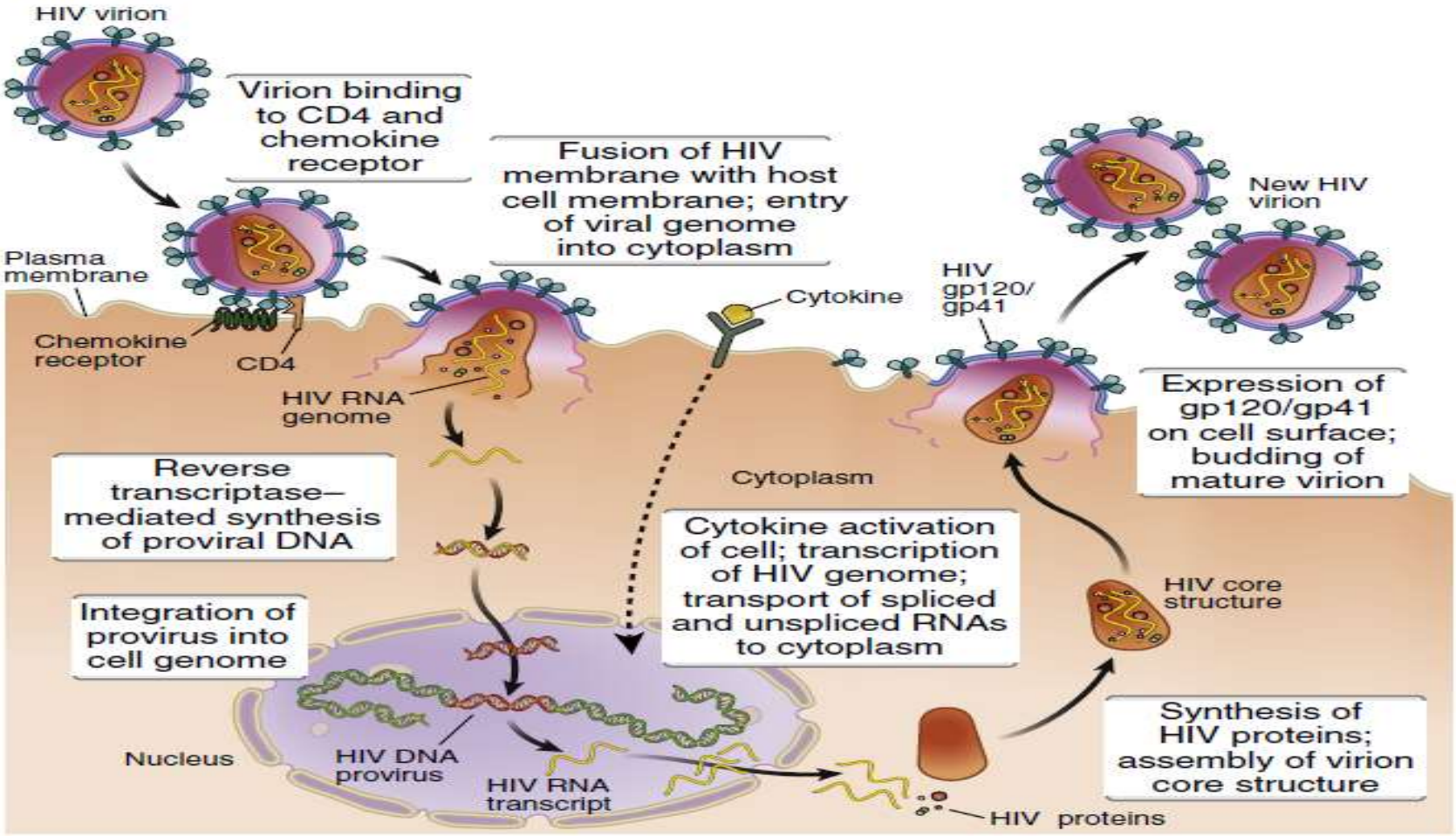


- **DNA synthesis :** **Reverse transcriptase** is the enzyme responsible for the synthesis of the double DNA strand that is complementary to the RNA molecule of the viral genome. The parts at the ends of the genomic RNA are copied twice, so that at each end of the newly synthesized DNA there are specific sequences **called long terminal repeats** (LTR)

- **Integration :** The DNA is then transported to the nucleus and integrated into the host cell's genome with the help of viral integrase, which joins the ends of the LTR sequence for the host cell's DNA. In an integrated state, the viral genetic material is called a **provirus**. **A provirus is analogous to a cellular gene, and is transmitted to daughter cells after division**.

HIV
- replication -

- **Synthesis of new viruses - in "productive phase"** of infection viral DNA is transcribed into mRNA by cellular **DNA-dependent RNA polymerase**. The signals for viral RNA synthesis are found in the LTR sequences. Some of the newly synthesized viral RNA molecules are used as mRNA for the synthesis of viral proteins, while the rest are incorporated into the genome of new virions.
- **Assembly and release of virions**



HIV
-latency and reactivation-

- **Latent phase** - infected cells contain provirus but do not express viral RNA or viral proteins

After infection of lymphocytes with HIV and integration of the provirus, the infectious process can be arrested and reactivated explosively after a certain time by another stimulus

More precisely, in case of activation of an infected T lymphocyte, macrophage or dendritic cell to some external stimulus (infection), the cell responds by transcribing more of its own genes and producing cytokines

An adverse consequence of the 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

HIV
-latency and reactivation-

HIV proviruses contain promoters that induce the expression of viral genes when HIV-infected cells are stimulated with antigen or infected with other microorganisms.

HIV expresses macromolecules that regulate the expression of the viral genome and function as soluble factors:

- ***Tat protein (transcription activator)*** accelerates and enhances transcription of integrated viral DNA with the help of host RNA polymerase

- ***Rev protein (regulator of viral gene expression)*** promotes the transport of viral RNA from the nucleus to the cytoplasm

How does HIV evade the host's immune response?

- *Nef protein (negative effector)*
  it reduces the expression of MHC class I molecules on the cell surface, blocks apoptosis, and enhances virus infectivity

- *Vif protein (viral infectivity factor)*
  cancels the inhibitory effects of cellular components

- *Vpu protein (viral protein)* promotes the destruction of CD4 and affects the release of virions

How does HIV evade the host's immune response?

- Viral gene products can be relatively invisible to the immune system
-
- A virus can mask or change its antigenic repertoire - **antigenic variation**
- The virus primarily replicates in lymph nodes, where immune system cells specific for virus antigens do not migrate freely

HIV
-antigenic variation-

HIV evades the host's immune response by altering large surface antigens

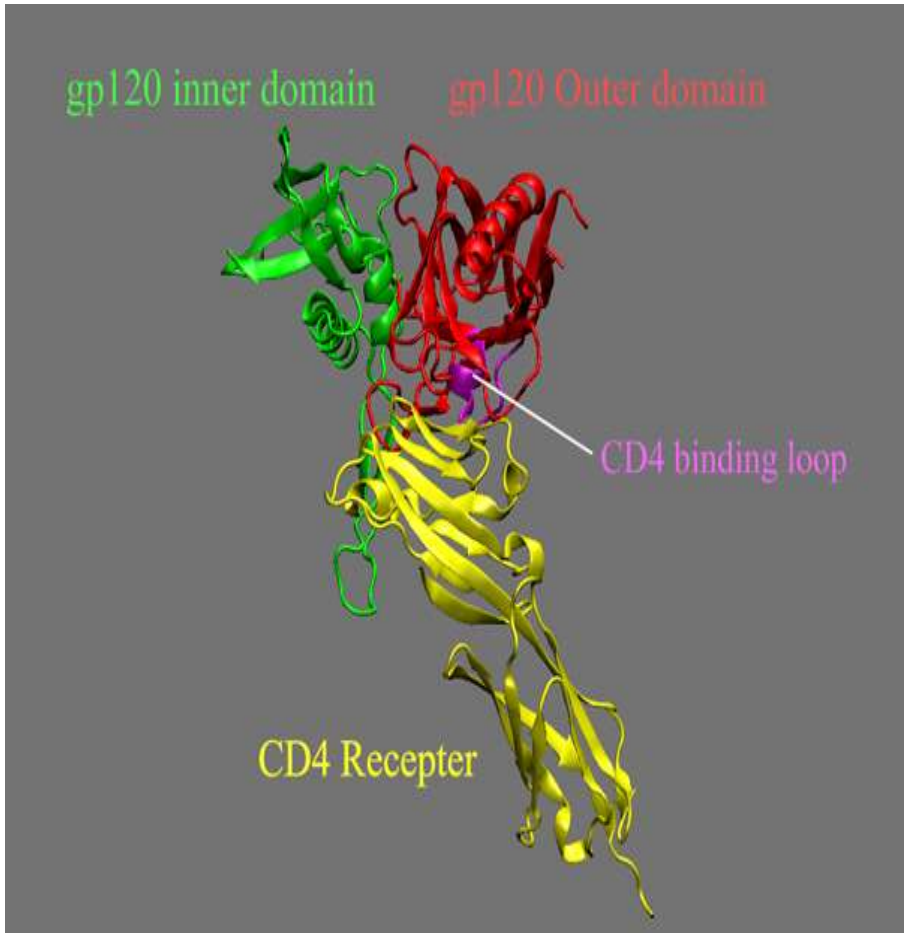
- Genes encoding internal viral proteins (gag and pol) show relative stability

- **The env gene is subject to numerous mutations that induce variability in the surface glycoproteins gp41 and gp120**

HIV
-antigenic variation-

- Sequences of gp120 surface glycoprotein, involved in interactions with cell receptors must be genetically conserved

- Conserved sequences can be hidden and thus protected from neutralizing antibodies by **carbohydrate chains and hypervariable regions**

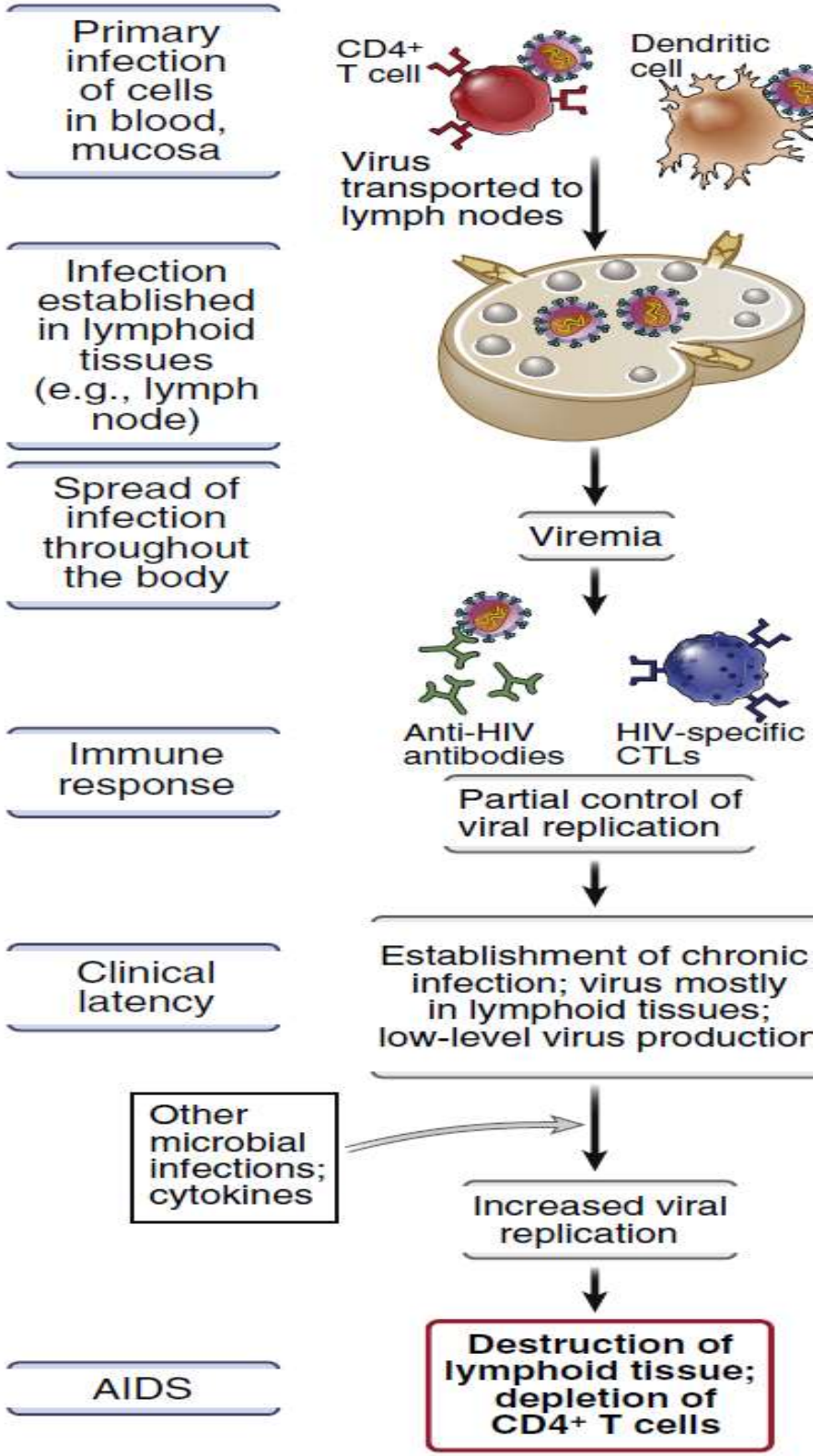


HIV
- disease pathogenesis -

Infection and depletion of helper T lymphocytes

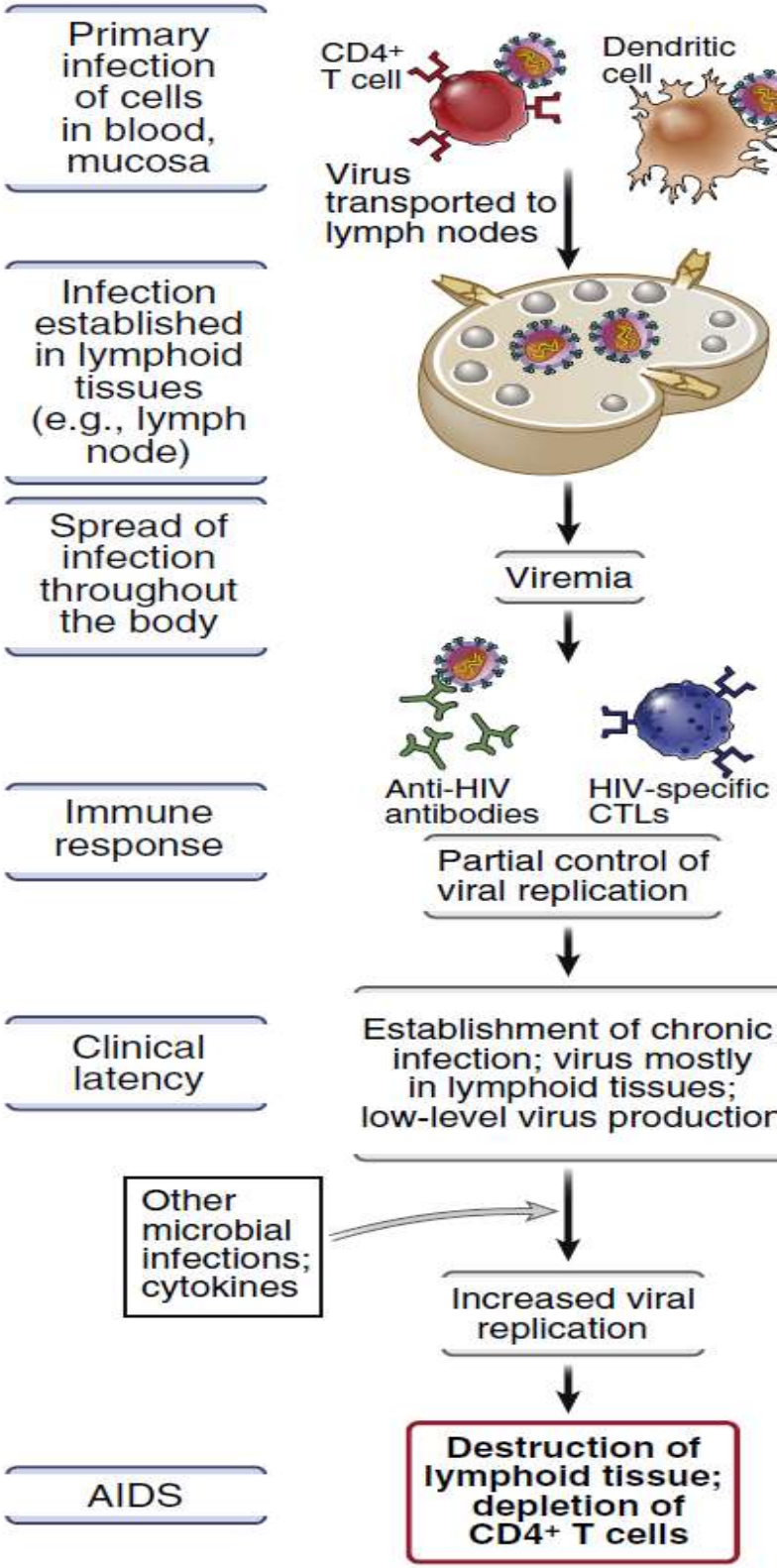
- **The CD4 molecule can also be found on the membranes of other cells:** monocytes and macrophages (disorders of phagocytosis), NK cells, some B lymphocytes, glia cells and Langerhans cells (important for the establishment of infection)

- **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



Tissue damage
Acute HIV infection

- The virus infects cells that express the CD4 molecule in local mucous membranes, and then rapidly establishes infection in local lymphatic tissues.
- During the next few days, **local replication** is limited to cells present at the site of viral entry. In most cases, the number of local susceptible cells decreases and the infection "dies" 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 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.



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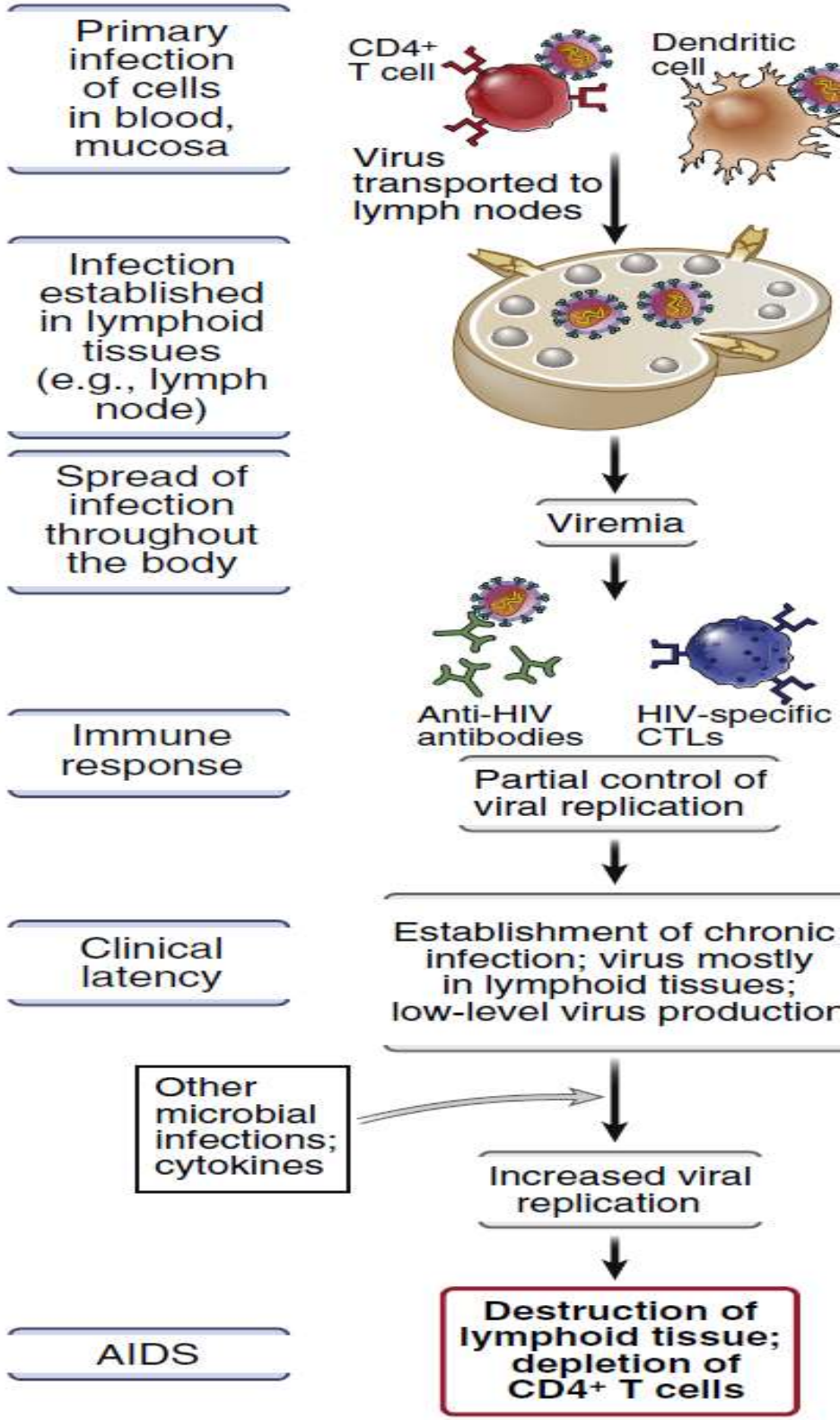
After infection at the site of entry, the virus spreads rapidly systemically, to distant organs of the lymphatic tissue and the central nervous system (CNS). In this phase, the virus shows the highest level of replication in the entire course of the disease and appears in genital secretions - the possibility of transmission is high

In the first weeks after the onset of 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, the 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



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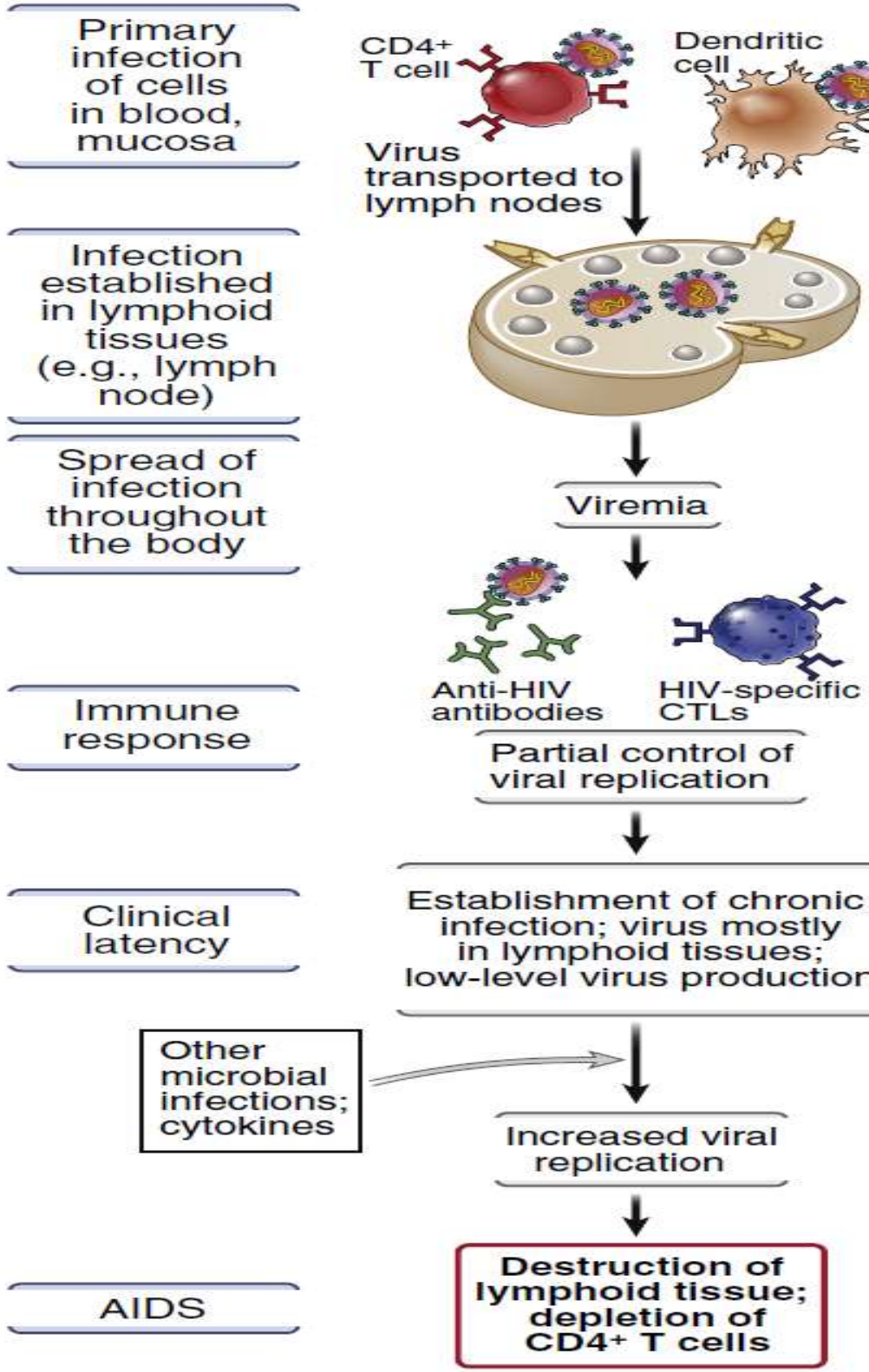
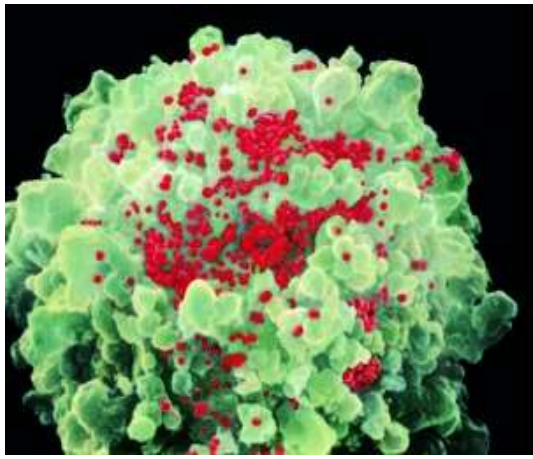
After the first few months of infection, a balance is established between **viral replication, effector immune mechanisms and available cells for viral replication**, and the infection enters a **latent phase** during which the infected person is symptom-free.

After the initial phase of HIV infection, viral replication is limited mainly to lymphoid organs where the main target is activated CD4+ T lymphocytes and 99% of viral replication takes place in them



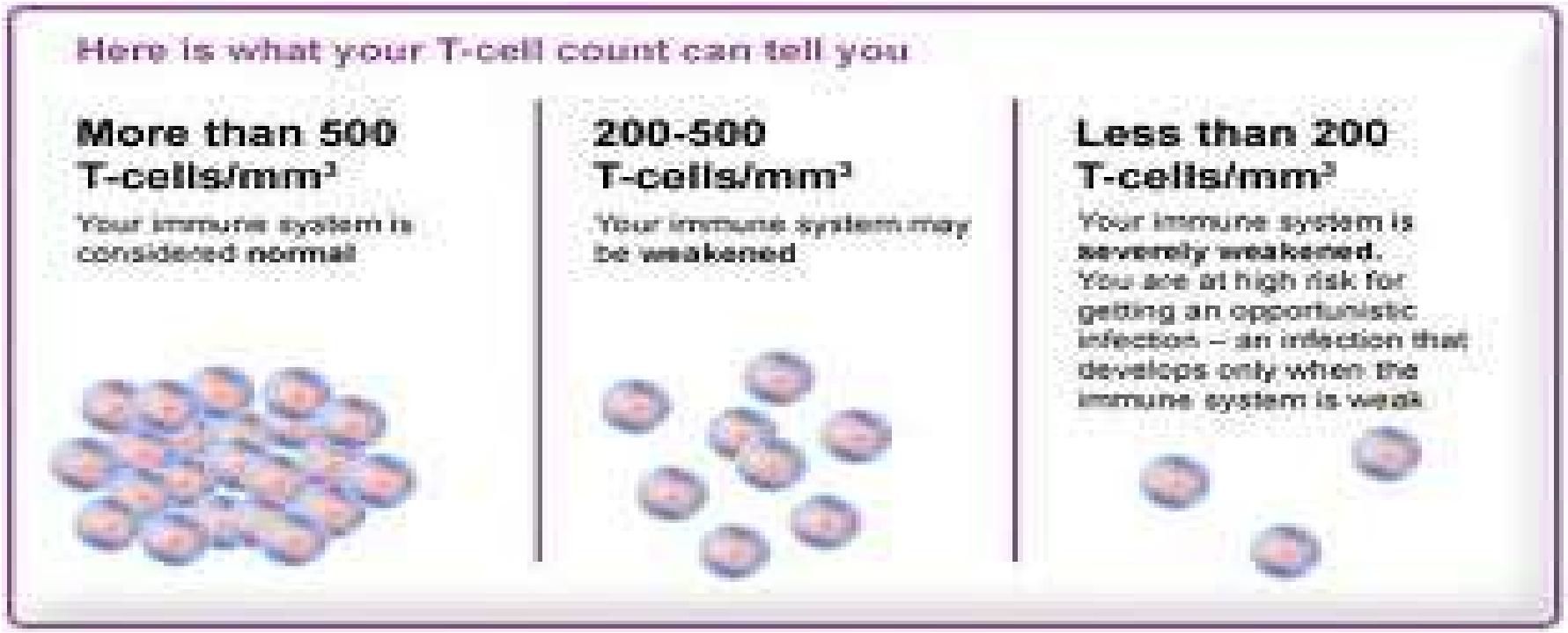
Tissue damage



- HIV-infected cells can be directly destroyed in the viral process replication or effector specific immune response mechanisms (cytotoxic T lymphocytes or antibody-dependent cytotoxicity)

- The loss of the CD4+ T cell population affects the development of 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



- People with **advanced AIDS** usually have **fewer than 200 CD4+ T lymphocytes per mm³**

- The risk of infections is greatest in the **terminal stages of AIDS**, when the CD4+ T lymphocyte count falls **below 50 cells/mm³**

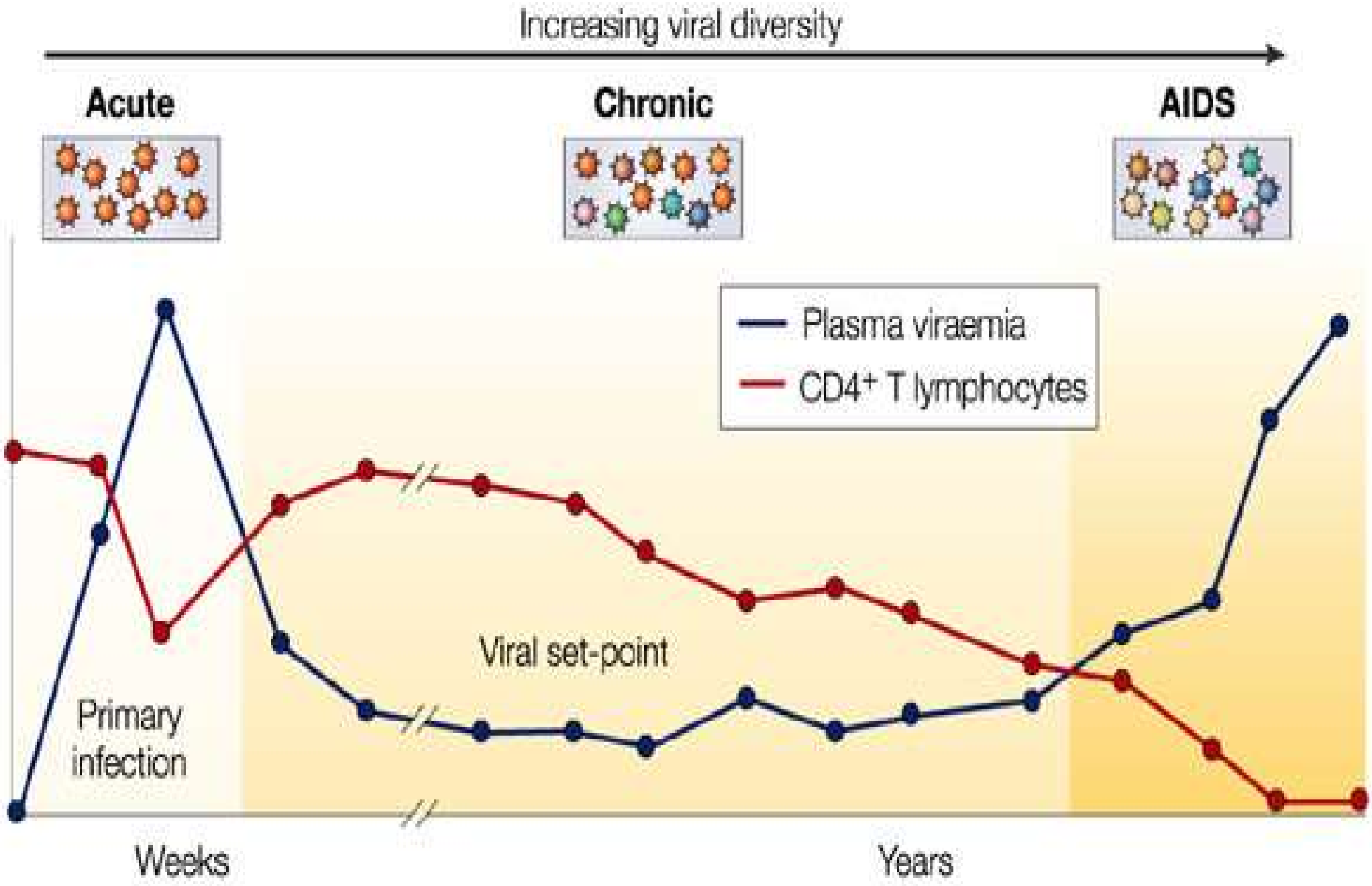
- Serial measurements of the number of CD4+ T lymphocytes serve to assess the risk of infections and are a guide for the implementation of antiretroviral therapy

Level of viral replication

Number of HIV RNA copies in plasma

Disease progression

People with **high levels of viral RNA** (10^5 copies/ml or more) are at **greater risk of disease progression** within a few years, while infected people with lower levels ($<10^4$ copies/ml) remain asymptomatic for 10 years or longer


Increasing viral diversity
Acute
Chronic
AIDS
Plasma viraemia
CD4+ T lymphocytes
Viral set-point
Primary infection
Weeks
Years

Aquired Immunodeficiency Syndrome (AIDS)

Two characteristics make AIDS unique among infectious diseases:

it is a deadly disease

and most of its devastating symptoms are not the result of the direct action of the causative agent of the disease

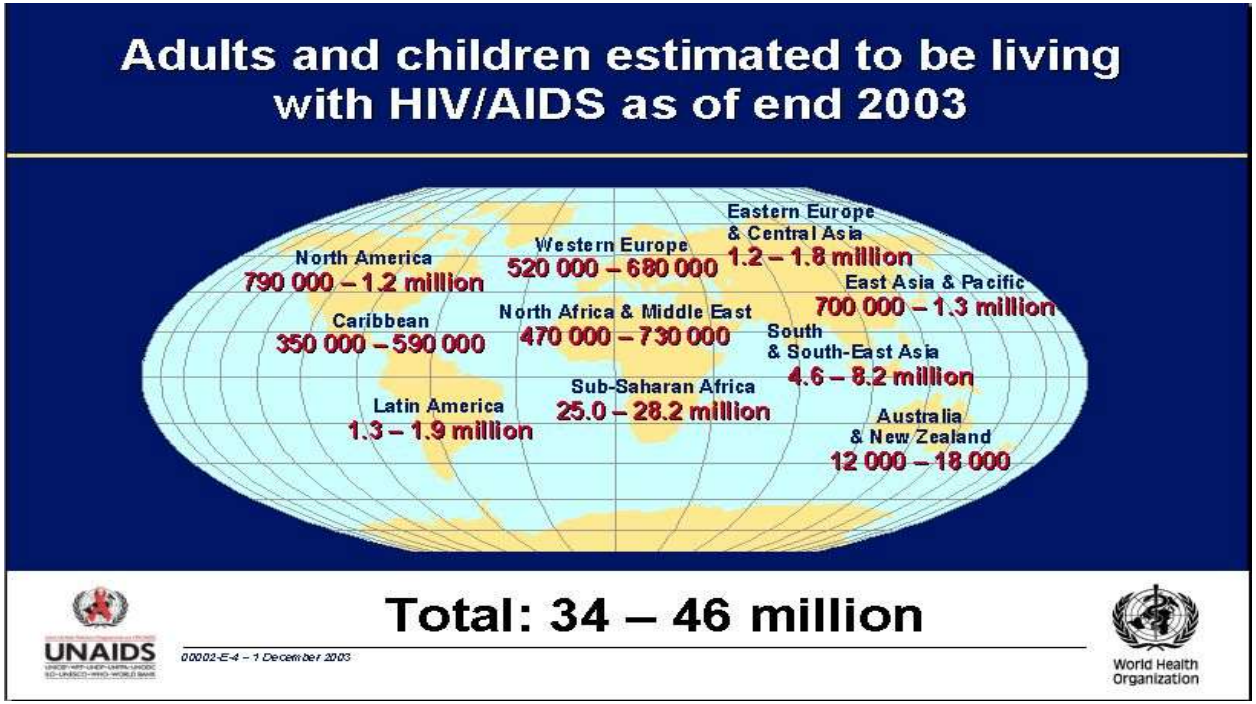
AIDS is a set of clinical diseases, primarily opportunistic infections and malignancies, which occur as a result of the destruction of the immune system by HIV.

The syndrome is a terminal manifestation of HIV infection that occurred many years earlier (10 or more years).

AIDS - PANDEMIC

- It is estimated that there are more than 34 million people infected with HIV in the world, about 70% are in Africa and 20% in Asia

- The disease has been attributed to the death of more than 30 million people worldwide, and the annual death rate now reaches around two million.

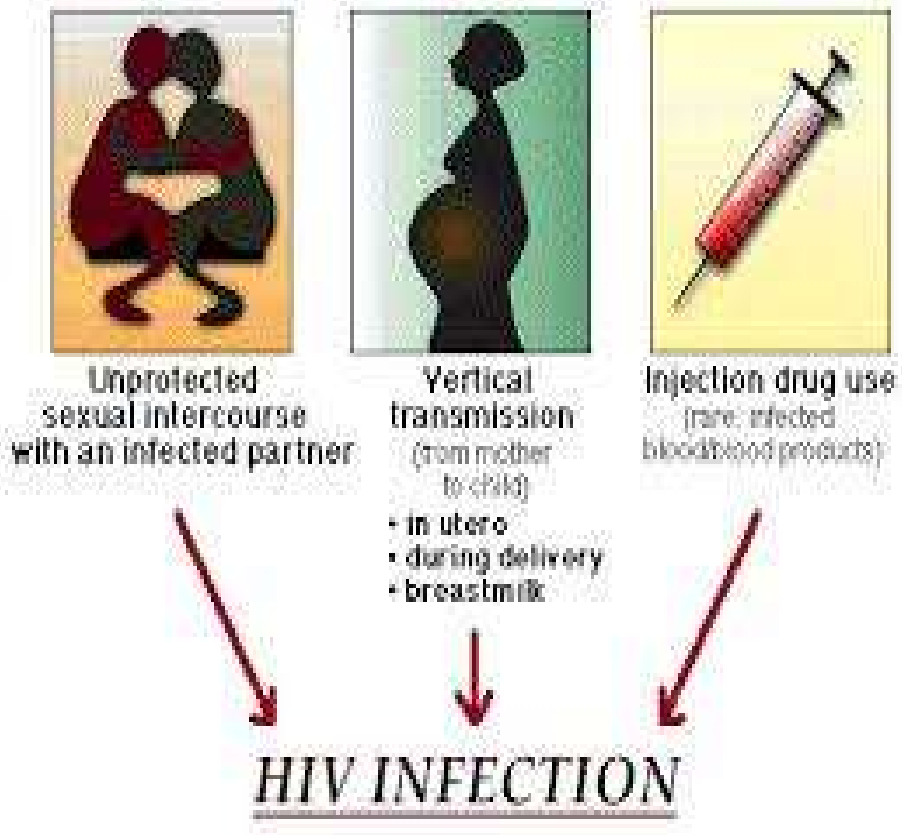


- Unfortunately, only 10% of people living with HIV in the world have access to antiretroviral therapy

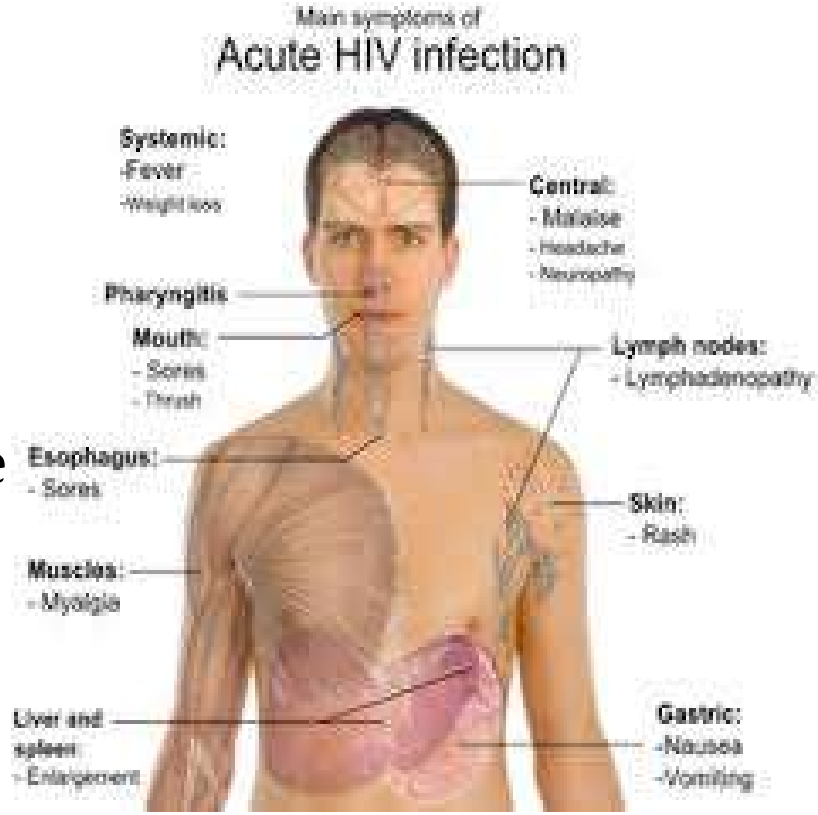
HIV transmission

HIV is primarily transmitted by direct inoculation of infected blood or body fluids into the host's body

- Sexual contact - 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 through infected blood and blood products
- Intravenous drug abuse
- Vertical transmission
- Occupational exposure – healthcare workers



Early acute HIV infection



- In 50 to 90% of people, the acute disease occurs 2 to 4 weeks after infection
- In most cases, the only symptoms are fever and mild sore throat
- Fewer patients may have fever, myalgias, lethargy, pharyngitis, arthralgias, lymphadenopathy, maculopapular rash, or aseptic meningitis.
- The acute illness usually lasts from 3 to 14 days and, as a rule, complete recovery occurs, even in patients with neurological complications.

Diagnosis of HIV infection

- **By determining the viral RNA, using the PCR method**, HIV infection can be detected early in the course of the infection, but due to the cost, it is not used as a screening test, unless the doctor suspects an acute infection.

- Instead, HIV infection is usually diagnosed by **detecting circulating antibodies to viral antigens**

Any presence of anti-HIV antibodies must be considered an active infection that can be transmitted to others

Diagnosis of HIV infection -serological tests-

Specific anti-HIV antibodies usually appear 6 to 12 weeks after infection

In rare cases, infected persons do not develop antibodies for several months or years after exposure to the virus - **false-negative HIV serological tests**

- In addition, some patients in the terminal stages of AIDS may have **negative serological tests** (probably due to **severe B lymphocyte dysfunction**)

Diagnosis of HIV infection -serological tests-

ELISA test:

- very sensitive test (> 99%), but not completely specific, so false-positive results are possible - verification of a positive finding is necessary

Western blot:

- sensitive and specific method for the detection of anti-HIV antibodies, but it is expensive and requires a lot of time for basic screening needs

Diagnosis of HIV infection PCR test

A sensitive and specific method for early detection of infection when specific anti-viral antibodies have not yet appeared

It is most often used for:

- assessment of the need and effectiveness of antiretroviral therapy
- identification of HIV-infected children born to HIV-positive mothers, when the presence of maternal antibodies may complicate serological diagnostic tests

Diagnosis of HIV infection
-other diagnostic tests-

- **P24** is an antigenic protein of the viral core, and its presence indicates active viral replication. However, in an already established infection, this antigen cannot be detected in the serum of all patients and is therefore less useful

- 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.

Consequences of HIV infection

HIV infection is a state of activation of the immune system with high turnover between the virus and CD4+ T lymphocytes, which occurs daily until the lymphocyte reserve of the organism is depleted.

The condition of patients during the progression of HIV infection and the decision to start antiretroviral therapy are routinely evaluated in three ways:

- clinical assessment of conditions related to HIV infection or AIDS
- by determining the number of CD4+ T lymphocytes
- by quantifying the level of viral RNA

Progression of infection to AIDS

- **Latent period** (from a few months to more than 15 years) - infected people usually do not have any symptoms of the disease and feel healthy

Possible manifestations:

- localized or generalized lymphadenopathy
- recurrent mucocutaneous candidiasis
- aphthous ulcers in the mouth, hairy leukoplakia (EBV)
- hematological cytopenias
- viral hepatitis
- skin changes - dry skin or itching, seborrheic dermatitis, eczema, folliculitis, psoriasis, herpes zoster

Clinical manifestations of AIDS

- "AIDS-associated diseases" are the most common **infections caused by intracellular pathogens** 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

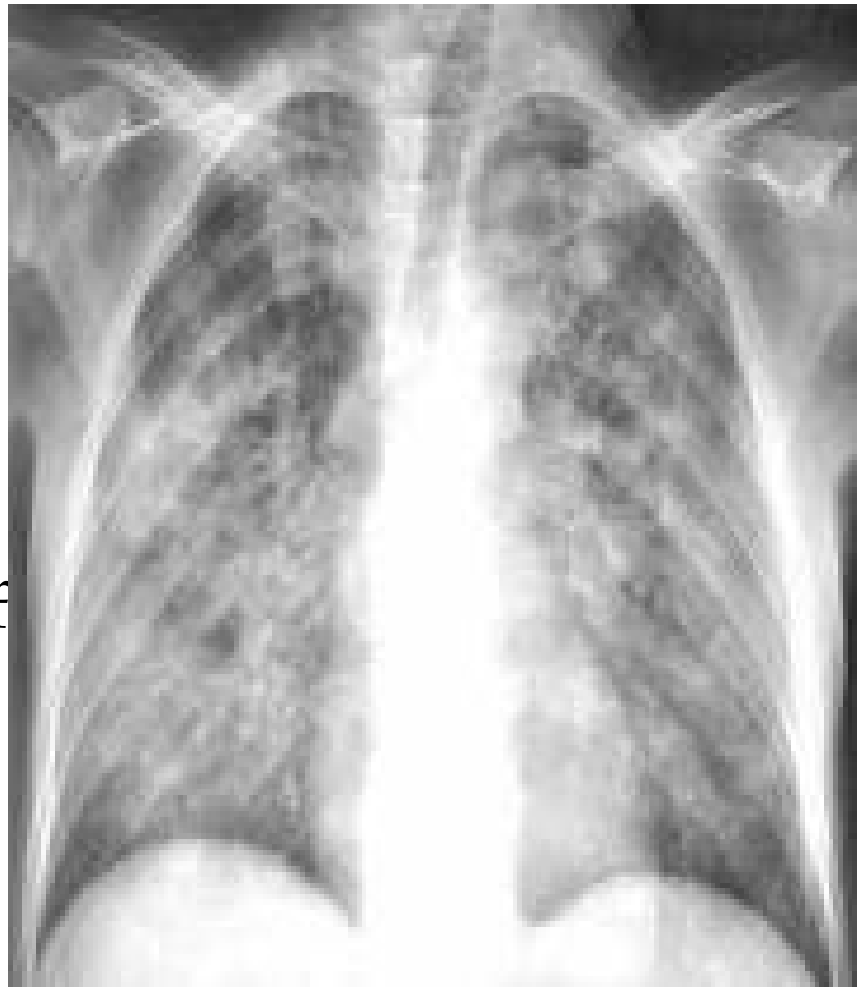
...

- Kaposi's sarcoma
- Primary brain lymphoma
- Non-Hodgkin lymphoma of V 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

Pneumonia caused by Pneumocystis jiroveci (PCP)



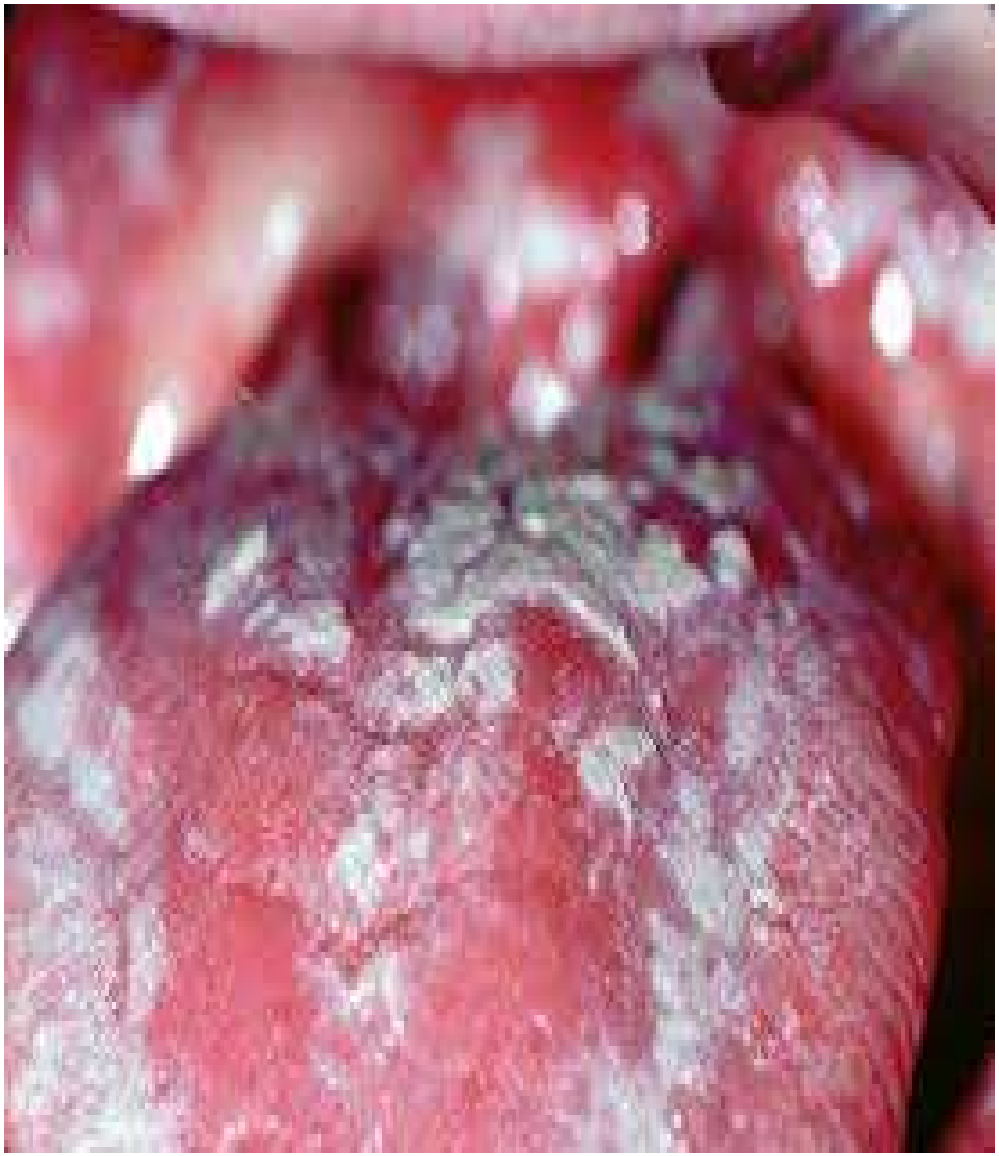
- The most common opportunistic infection associated with AIDS and occurs in 25 to 60% of patients
-
- Typical symptoms are fever, cough and shortness of breath
- It is associated with a mortality rate of 10 to 20% of cases that develop irreversible respiratory failure.
- The use of specific antiretroviral therapy and anti-PCP therapy has improved quality of life and length of survival

Gastrointestinal infections

- Oral and pharyngeal **candidiasis**, esophageal candidiasis, accompanied by pain and difficulty swallowing with consequent weight loss

- **CMV** typically causes disseminated disease accompanied by viremia. Involvement of the large intestine can result in intense abdominal pain and diarrhea

- Gastrointestinal symptoms can also be caused by malignancies such as **Kaposi's sarcoma** or **lymphoma** of the stomach or colon



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:

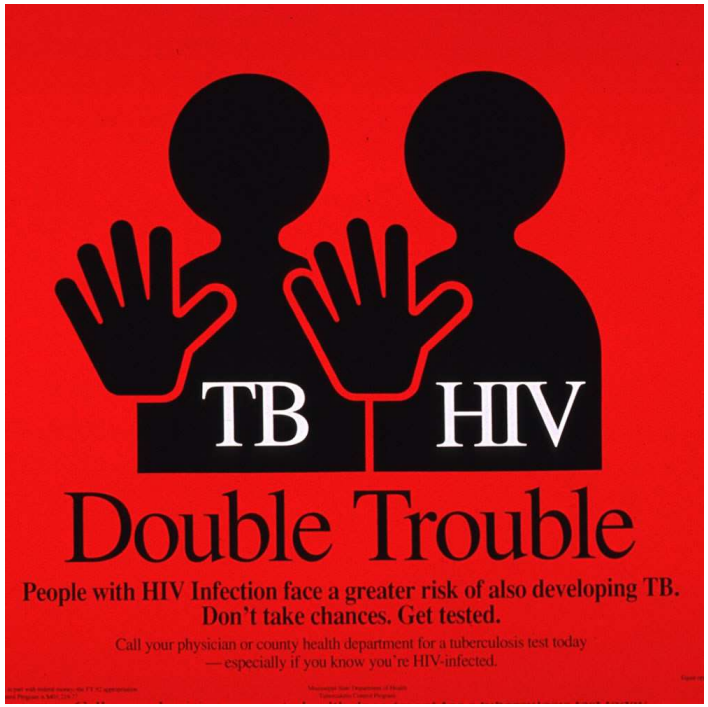
- CMV and other viruses
- intestinal Gram-bacteria such as *Salmonella* and *Shigella* spp. (with accompanying bacteremia)
- hospital infections - *Clostridium difficile*
- mycobacterial infections (especially *Mycobacterium avium* complex) primarily of the small and large intestine, followed by malabsorption and diarrhea
- intestinal parasites such as *Giardia, Isospora, Cryptosporidium* and *Microsporidium* spp.

Mycobacterial and fungal infections

- **Tuberculosis** of the lungs that can spread and manifest as lymphadenitis, hepatitis or meningitis

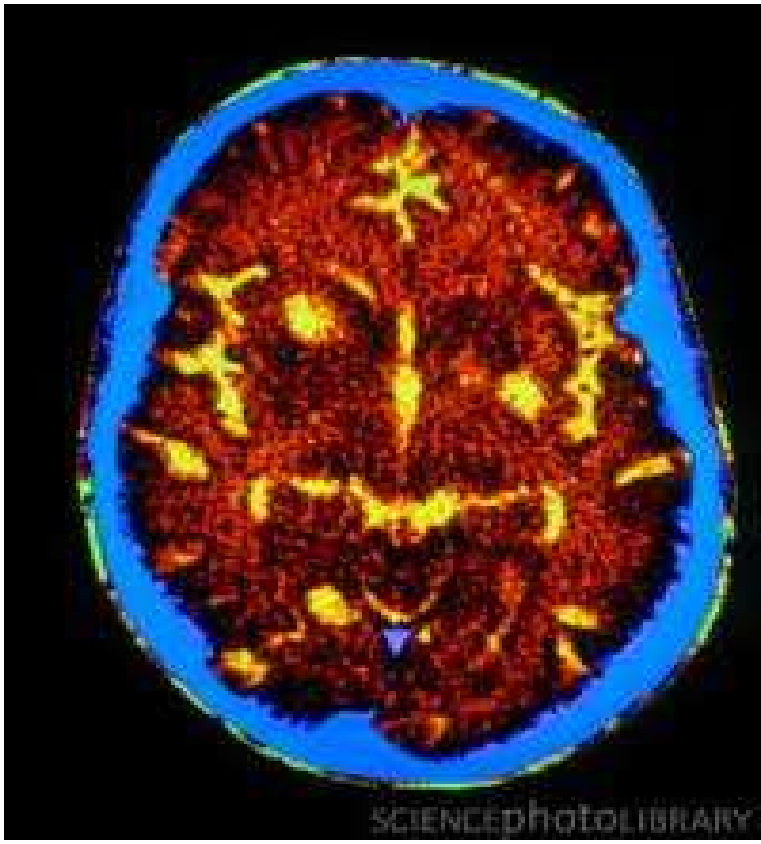
- Disseminated infection caused by *M. avium* complex (MAC): fever, night sweats, weight loss, splenomegaly, hepatomegaly, and diarrhea

- Similar symptoms are seen in patients with disseminated fungal infections, such as histoplasmosis and coccidiomycosis.



Infections of the nervous system

- ***Cryptococcus neoformans*** – meningitis



- Reactivation of infection with the parasite **T. gondii** typically causes multifocal brain infection. Patients may experience headache, confusion, or seizures

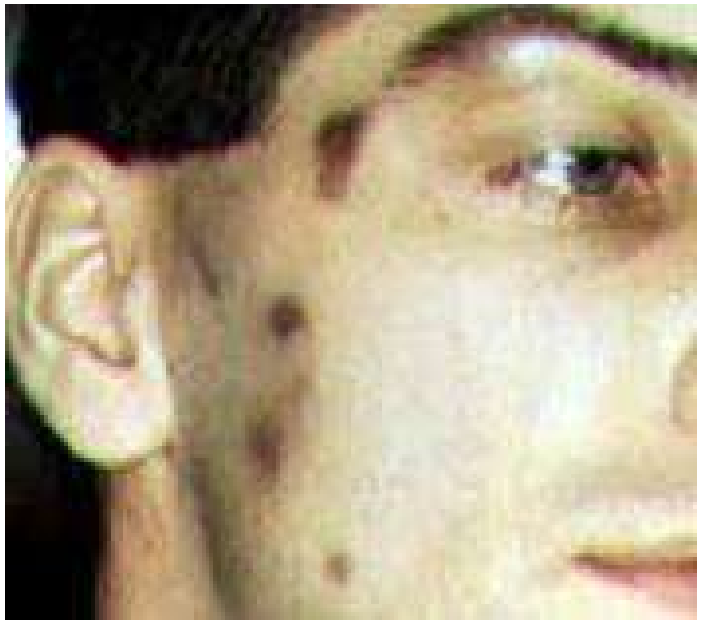
- **CMV** causes retinitis and occasionally encephalitis

Direct manifestations of HIV infection

- **HIV nephropathy -** proteinuria, nephrotic syndrome and renal failure
- **Myopathy and myositis**
- **Cardiomyopathy**
- **Weight loss**

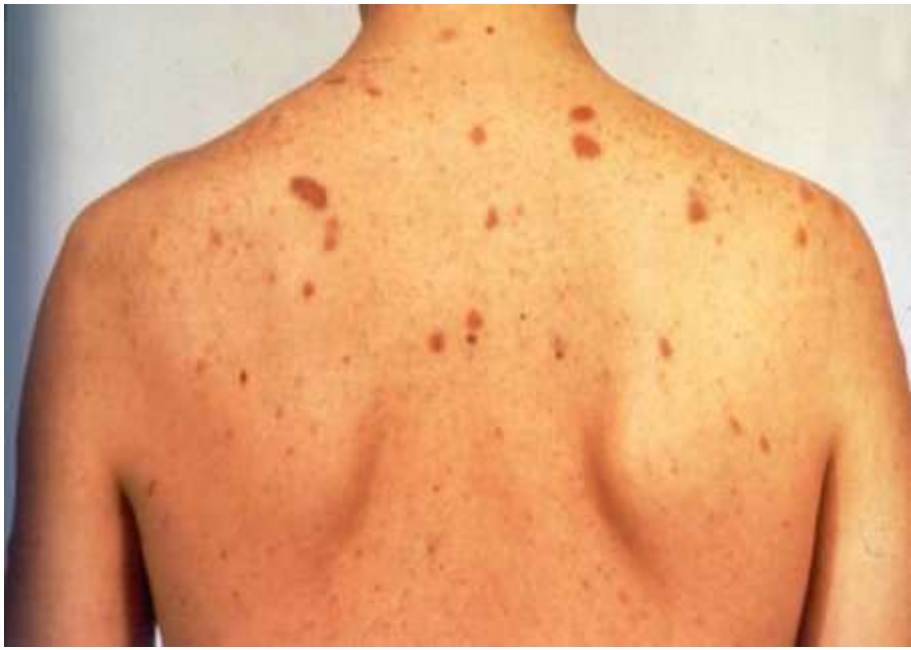
Oncological manifestations of advanced AIDS

- **Kaposi's sarcoma** (HHV-8) - a localized skin lesion without significant symptoms. Severe cases of Kaposi's sarcoma manifest as widely disseminated lesions, with involvement of the lymph nodes, gastrointestinal tract, and lungs.



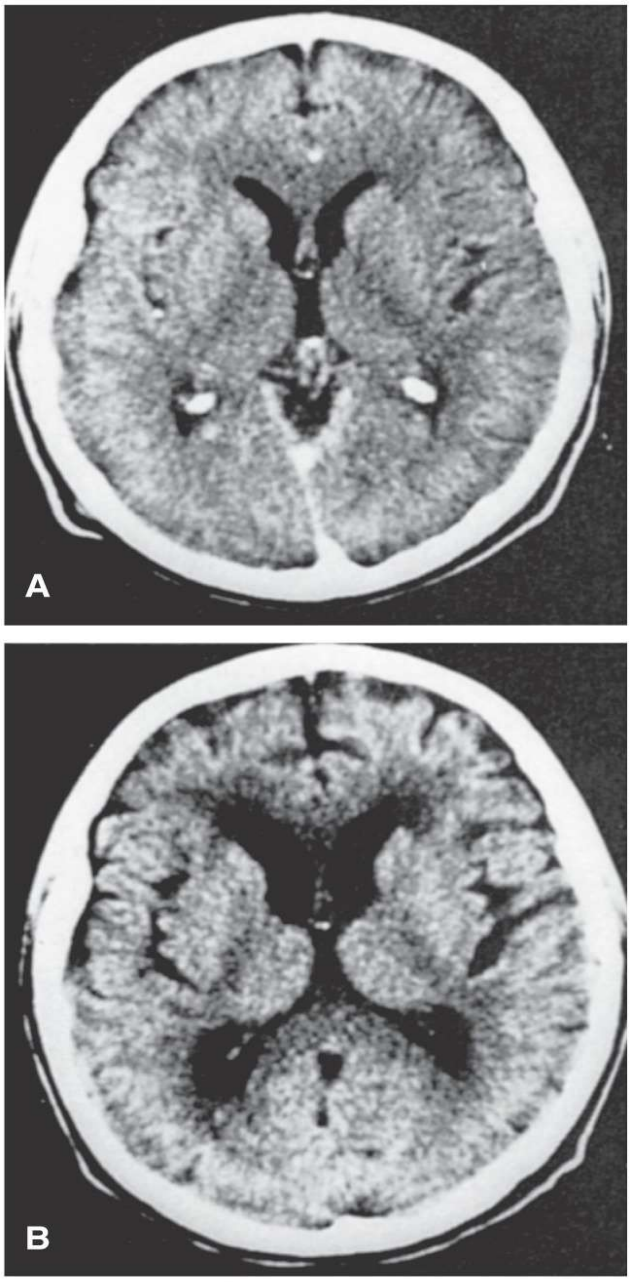
- Hodgkin's and non-Hodgkin's lymphoma (EBV infection)

- Malignant transformations associated with HPV infection



Neurological manifestations in advanced AIDS

- The most common neurological problems are caused by HIV itself
- **Acute primary infection** may be associated with complications such as **aseptic meningitis, encephalitis, myelitis or inflammatory neuropathies** such as ***Guillain-Barré*** **syndrome.**
- In the later stages of the disease, patients may have **peripheral neuropathies, motor or sensory neurological deficits**
- **The most common form of neurological disease is HIV-associated encephalopathy followed by progressive dementia**



HIV infection in children

- 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%

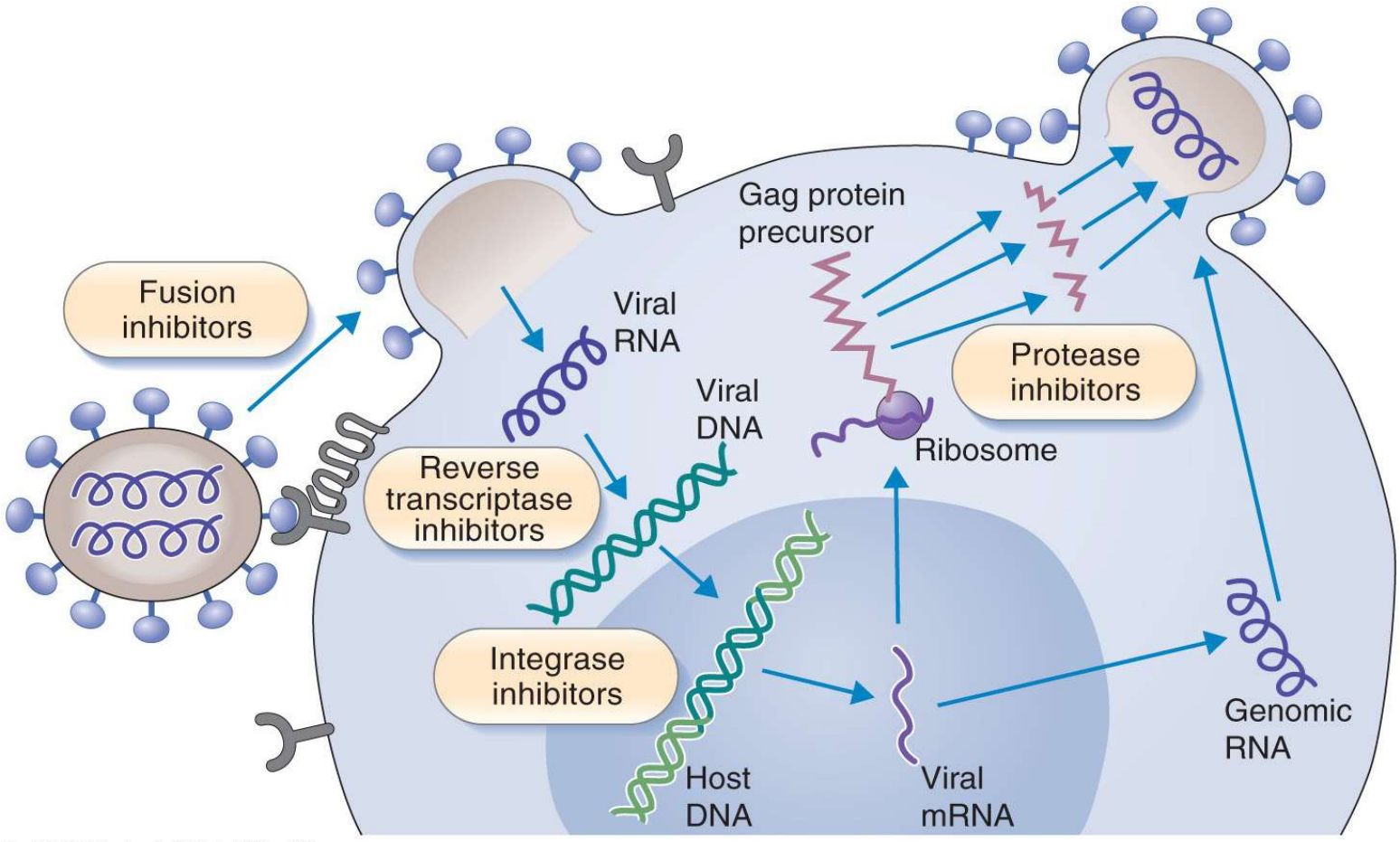
- HIV infection in children has a similar course with progressive immunodeficiency, recurrent opportunistic infections and neurological manifestations. However, disease progression can be much faster in infants

Treatment of HIV positive patients

- Antiretroviral therapy has changed HIV infection from a fatal to a chronic disease
- Education of the patient and the environment
- Support

Antiretroviral therapy

Effective drugs that act at different points in the viral life cycle: binding to CCR5 (coreceptor), viral envelope fusion, retrovirus-specific DNA polymerase, integration into the host genome, and viral protease



Antiretroviral therapy

Treatment of HIV infection involves a combination of drugs with the aim of achieving synergism and delaying the emergence of resistance

“Highly active antiretroviral therapy " (HAART)

for example. two reverse transcriptase inhibitors and one protease inhibitor

Prophylaxis of infections - antibiotics

HIV - Prevention

- The best approach to controlling AIDS is to prevent HIV transmission
- Regular screening of persons at risk of HIV infection
- HAART therapy – reduced risk of transmission
- Development of an effective vaccine

Vaccines

Are there other ways to acquire immunity (memory lymphocytes)?

Immunity can be acquired through infection (natural route) or vaccination.

Vaccines are weakened or dead microorganisms (or their parts) that induce the development of memory lymphocytes.

As vaccines enable the acquisition of immunity (memory lymphocytes) without getting sick, they represent particularly suitable protection against very dangerous infectious diseases.

First vaccine: against smallpox

Edward Jenner (1749 - 1823)

1796: James Phipps receives the first vaccine1798: “An Inquiry into the Causes and Effects of the Variolae Vaccinae, a Disease Discovered in some of the Western Counties of England, particularly Gloucestershire, and Known by the Name of the Cow Pox”

The first bacterial vaccines

Louis Pasteur (1822 – 1895)

1879: vaccine to protect poultry against fowl cholera

1881: vaccine to protect cows from anthrax

1885: Rabies vaccine
Joseph Mayer

Immune response induced by vaccines

serum antibodies (IgG and IgM)

for extracellular bacteria (opsonization, phagocytosis, complement), bacterial exotoxins (neutralization) and viruses

mucosal antibodies (IgA)

for extracellular bacteria (adherence inhibition) and viruses

cellular immunity (T lymphocyte - macrophage)

for intracellular bacteria and viruses

The ideal vaccine

- **Effective -** highly immunogenic and provides complete and long-term (lifelong) protection
- **Natural -** painless and one-time application
- **Safe -** no side effects
- **Stable**
- **Cheap**

Vaccine safety - adverse reactions

Toxic/inflammatory reactions

local, systemic

infections

immunopathological reactions

hypersensitivity reactions

Vaccine safety - adverse reactions

Limitations and problems with vaccines
Inactivated vaccines - microorganisms are not adequately killed
Attenuated microorganisms can transform into wild type and regain virulence (eg polioviruses types 2,3)
Inclusion of toxic material (eg typhoid, whooping cough)
Contamination with animal viruses
Egg protein contamination (hypersensitivity, allergy)
Cross reaction with one's own (autoimmune diseases)
Limitations and problems on the part of the patient
Immunodeficiency (live microorganisms can cause serious diseases)
Local inflammatory reaction, often to an adjuvant
Vaccine hypersensitivity (eg tetanus)
Interference between vaccines given at the same time (not always)

Types of vaccines

1. Live vaccines

- **attenuated**
- **virulent**
- **recombinant**

2. Dead vaccines

- **Inactivated**
- **whole microorganisms**
- **Subunit**
- **purified**
- **synthetic**
- **recombinant**

3. DNA vaccines

Types of vaccines

Type of vaccine (antigen)	Examples
Live vaccines	Smallpox, measles, mumps, rubella, poliomyelitis (Seibin vaccine), yellow fever, varicella, BCG, cholera, rotavirus
Dead (inactivated) vaccines containing whole pathogens	Rabies, influenza, poliomyelitis (Salk vaccine), hepatitis A, pertussis, typhoid, cholera
Dead (subunit) vaccines containing antigenic fragments (protein, polysaccharide, conjugated)	Meningococcus, Pneumococcus, H. influenzae, hepatitis V, tetanus, diphtheria, pertussis
DNA vaccines	*Influenza, HIV

*Vaccines in the experimental phase of testing

Characteristics of induced immune response

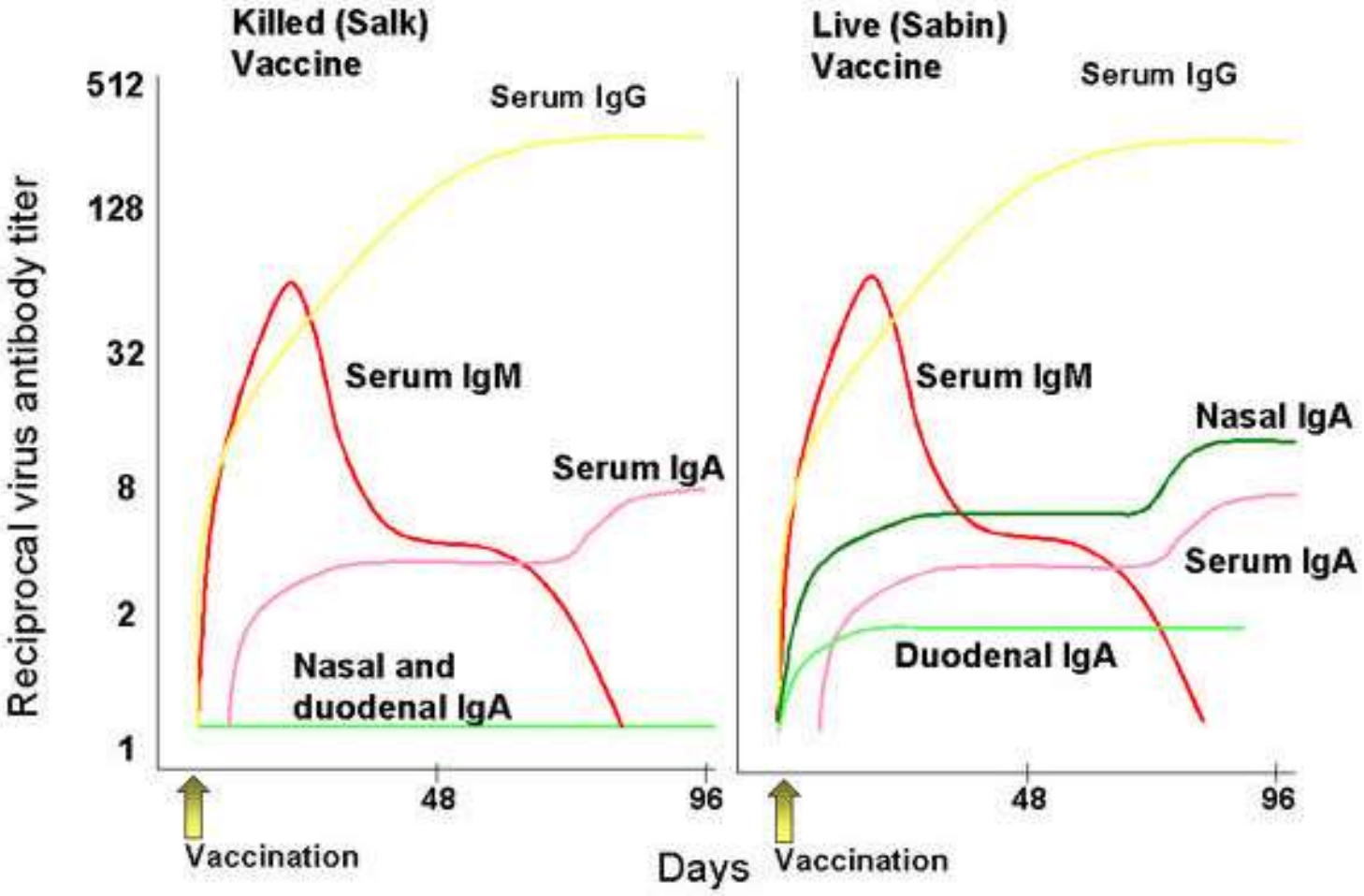
Live vaccines

- good humoral and cellular immune response
- good immune memory
- the immune response that follows a natural infection

Dead vaccines

- good humoral immune response
- variable: cellular immune response and immune memory

Characteristics of induced immune response



Live attenuated vaccines

They contain live, but weakened microorganisms that have lost their virulence, but have preserved their antigenicity

advantages

- activate both humoral and cellular immune responses
- they are given to mimic the natural route of infection
- stimulate interferon production
- good and long-lasting protection
- a small inoculum is sufficient
- no adjuvant is required

disadvantages

- attenuation and safety problem
- possibility of return of virulence
- interference with virulent viruses
- immunodeficiencies and pregnancy!
- stability

Live virulent vaccines

The similar antigenic composition of related viruses enables the induction of a cross-immune response

- v. vacciniae/v. variolae
- simian/human rotaviruses

Live recombinant vaccines

Vector vaccines

- They contain live avirulent viruses (vectors) into which the gene of the virulent virus responsible for the synthesis of the protective antigen has been inserted.

- The gene responsible for the synthesis of the key antigen is cloned into the vector genome.

- The most common vectors: c. vacciniae and other pox viruses

- A vector with a recombinant genome is given as a vaccine
- In the vaccinated organism, the vector multiplies and synthesizes a sufficient amount of antigen
- Vaccines against HBV, HSV

Live recombinant vaccines

Deletion mutants

They contain live infectious viruses in which the gene responsible for virulence has been mutated or removed by recombinant techniques

Dead vaccines

inactivated vaccines, which contain whole inactivated microorganisms and subunit vaccines consisting of antigenic fragments of microorganisms.

advantages

- **it is made from a completely virulent microorganism**
- **stability**
- **security**

Disadvantages

- **type and duration of protection**
- **more doses are required**
- **adjuvant required**

Adjuvants

They increase the immunogenicity of antigens and are used as additives in inactivated vaccines

Adjuvants increase the effectiveness (antigenicity) of the vaccine by stimulating and activating Toll-like receptors

By themselves, some cytokines such as IL-1, IL-2 and IFN-γ may have adjuvant activity

Inactivated vaccines

By applying physical or chemical procedures, the infectivity of viruses is destroyed, but their immunogenicity is preserved

Deficiency:

They stimulate the production of neutralizing antibodies, but in a lower titer than live vaccines

They induce a weak cellular immune response

They do not induce a local immune response

Short-term immunity

Subunit vaccines

They contain purified and concentrated antigens of microorganisms

Advantage:
Harmless

Deficiency:
Weaker immunogenicity
They do not induce a cellular immune response

Subumit protein vaccines

Diphtheria
toxoid (anatoxin)
Corynebacterium diphtheriae

Tetanus
toxoid (anatoxin)
Clostridium tetani

Pertussis
toxoid (anatoxin), adhesins
Bordetella pertussis

Toxoids as antigens in vaccines

Toxoids are chemically and temperature-modified bacterial toxins that have lost their toxicity and **retained their antigenicity** (diphtheria, tetanus, pertussis).

Since toxoids have fewer antigenic determinants, effective immunization **requires repeated doses of vaccination** in childhood, as well as revaccination every 10 years throughout life.

Subunit polysaccharide vaccines

In the prevention of infections caused by encapsulated bacteria

Composition: capsule polysaccharides

Haemophilus influenzae type b

Streptococcus pneumoniae 23 serotypes

Neisseria meningitidis 4 serotypes (A, C, Y, W135)

Polysaccharides as antigens in vaccines

- T-independent antigens: the immune response always has the characteristics of a primary response

Missing:

- stimulation of T lymphocytes, i.e. cooperation of T - B lymphocytes;
- change of Ig class;
- maturation of immune response affinity;
- immune memory;

Subunit conjugate vaccines

polysaccharide Ag + protein carrier

protein carriers:

- toxoid of C. diphtheriae toxin
- toxoid of C. tetani toxin

Subunit conjugate vaccines

In the prevention of infections caused by:

Haemophilus influenzae type b

illness 95-100%

Streptococcus pneumoniae - 7 serotypes

illness 95%

Neisseria meningitidis - 2 serotypes

illness 92%

Combination vaccines

They consist of a combination of several toxoids or inactivated pathogens that are administered simultaneously

MMR – Morbilli, Mumps, Rubella

DiTePer – Diphteria,Tetanus, Pertussis

Subunit recombinant vaccines

They contain purified antigens obtained by recombinant techniques

In vitro expression of individual genes for protective antigens

Insertion of the gene responsible for the synthesis of the key antigen into the genome of the expression vector (bacteria or yeast.

The cloned vector in vitro produces large amounts of antigen, which is then purified.

Purified antigen is given as a vaccine

Current vaccine against hepatitis V virus

DNA vaccines

- **Entry of NOT ANTIGEN but GENES of microorganisms**
  - construction of a DNA plasmid containing the gene responsible for the synthesis of the key Ag
  - by transformation, it is taken over by bacteria
  - multiplication of bacteria and amplification of plasmids
  - purification of plasmid DNA
  - entry into target cells (myocytes, keratinocytes)
  - transcription and translation of the target gene
- **The vaccinated organism develops an immune response to the heterologous protein produced by its own cells**

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DNA vaccines - advantages

- Plasmids can be obtained easily and in large quantities

  Cheap
- DNA is stable

  Storage and transport made easy
- DNA sequences can be easily altered

  Rapid response to changes in the infectious agent
- The antigen protein is post-translationally modified in our cells in the same way as during a natural infection

  Such an antigen is superior to antigens produced in expression vectors under in vitro conditions
- Mixtures of plasmids can be used

  Combination vaccines
- The plasmid does not replicate and only encodes the protein of interest

  There is no immune response to the vector microorganism
- The antigen is presented as part of the GHK molecule of the first class
- Induction of CTL

DNA vaccines

- Potential integration of plasmids into the genome
- Insertional mutagenesis
- Induction of an autoimmune response
- Induction of immune tolerance to an antigen

	VIRUSES	BACTERIA	FUNGI	PARASITES
Vaccines in general use	Poliomyelitis	Difteria Tetanus Pertussis		
	Morbili Mumps Rubella	BCG (in some countries)		
	Hepatitis B			
Vaccines that are given to people who are at increased risk of getting the disease	Influenza	BCG		
	Yellow fever	Typhus		
	Hepatitis A	Pneumococcus		
	Rabies	Meningococcus		
	Varicella-zoster virus	Haemophilus		
		Antrax		
Vaccines not yet available for use	Adenovirus	Staphylococcus	Candida	Malaria
	Rhinovirus	Streptococcus	Pneumocystis	Leishmania
	Herpes virusi	Gonococcus		Schistosomiasis
	Respiratory syncicial virus (RSV)	Syphilis		Filaria
	HIV	Leprosy		

Effectiveness of vaccines against common infectious diseases

Disease	Maximum number of cases (year)	Number of cases in 2014
Diptheria	206,939 (1921)	0
Measles	894,134 (1941)	72
Mumps	152,209 (1968)	40
Pertussis	265,269 (1934)	311
Polio (paralytic)	21,269 (1952)	0
Rubella	57,686 (1969)	0
Tetanus	1,560 (1923)	0
Hemophilus influenzae type B infection	~20,000 (1984)	134
Hepatitis B	26,611 (1985)	58

Reducing the incidence of infectious diseases against which effective vaccines have been developed

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