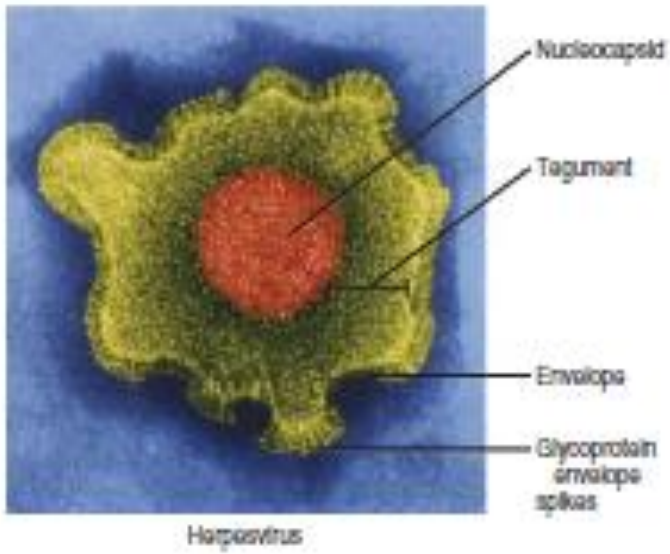
Virology chapter two

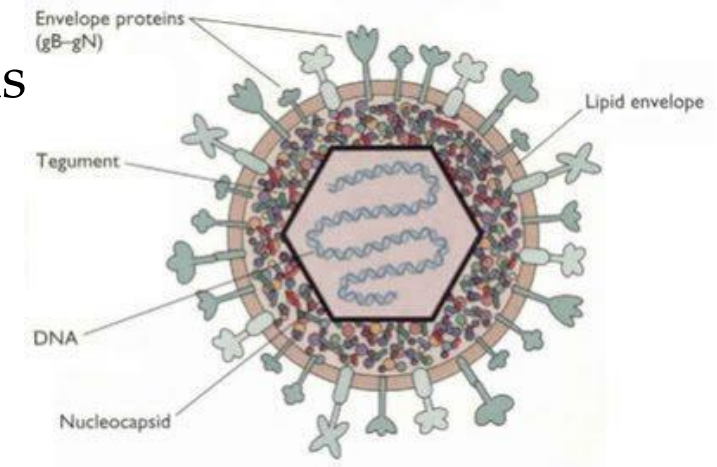
Herpesviruses
Viral hepatitis
Papillomaviruses
Rabies virus
Prions

Herpesviruses
family *Herpetoviridae*

Common characteristics

- **size** 180-200 nm
- **icosahedral** symmetry
- **double-stranded DNA**
- **envelope** containing viral glycoproteins
- **tegument (an unique feature of herpesviruses):** surrounds the nucleocapsid, contains structural proteins and enzymes necessary for rapid viral replication and establishing the initial infection
- human and viral ***micro*RNA**





members of family *Herpetoviridae*

Human herpesviruses

	Name	Mode of transmission	Primary infection site	Diseases	The site of latent infection
HHV-1	*Herpes simplex virus 1* **(HSV-1)**	Direct contact	Epithelial cells	Perioral lesions (cold sores), eye damage, encephalitis	Sensory ganglia neurons
HHV-2	*Herpes simplex virus 2* **(HSV-2)**	Direct contact, sexually transmitted disease	Epithelial cells	Genital herpes, neonatal herpes, encephalitis	Sensory ganglia neurons
HHV-3	*Varicella-zoster virus* **(VZV)**	Respiratory route , inhalation, direct contact	Epithelial cells	Chickenpox (primary infection) zoster (reactivation)	Sensory ganglia neurons
HHV-4	*Epstein-Barr virus* **(EBV)**	saliva	B cells, oral epithelium	Infectious mononucleosis (primary infection), B cell malignancies, (Burkitt lymphoma), nasopharyngeal carcinoma	B cells
HHV-5	*Cytomegalovirus* **(CMV)**	Direct contact , sexual transmission, congenital, transfusion, transplantation	Lymphocytes and monocytes	Mononucleosis, congenital infection, severe infection in immunosupressed (retinitis, gastroenteritis, pneumonia)	Monocytes, endothelial cells
HHV-6	*Human herpesvirus 6*	Direct contact , respiratory route	T cells	Roseola in children (primary infection), infections after transplantation (pneumonia)	T cells, monocytes, macrophages
HHV-7	*Human herpesvirus 7*	saliva, direct contact	T cells	Some roseola cases	CD4+ T cells
HHV-8	*Kaposi sarcoma virus, human herpesvirus 8*	saliva, blood?	B cells, peripheral blood mononuclear cells, oral epithelium	Kaposi sarcoma, B-cell lymphomas	B cells, tumor cells infected with the virus

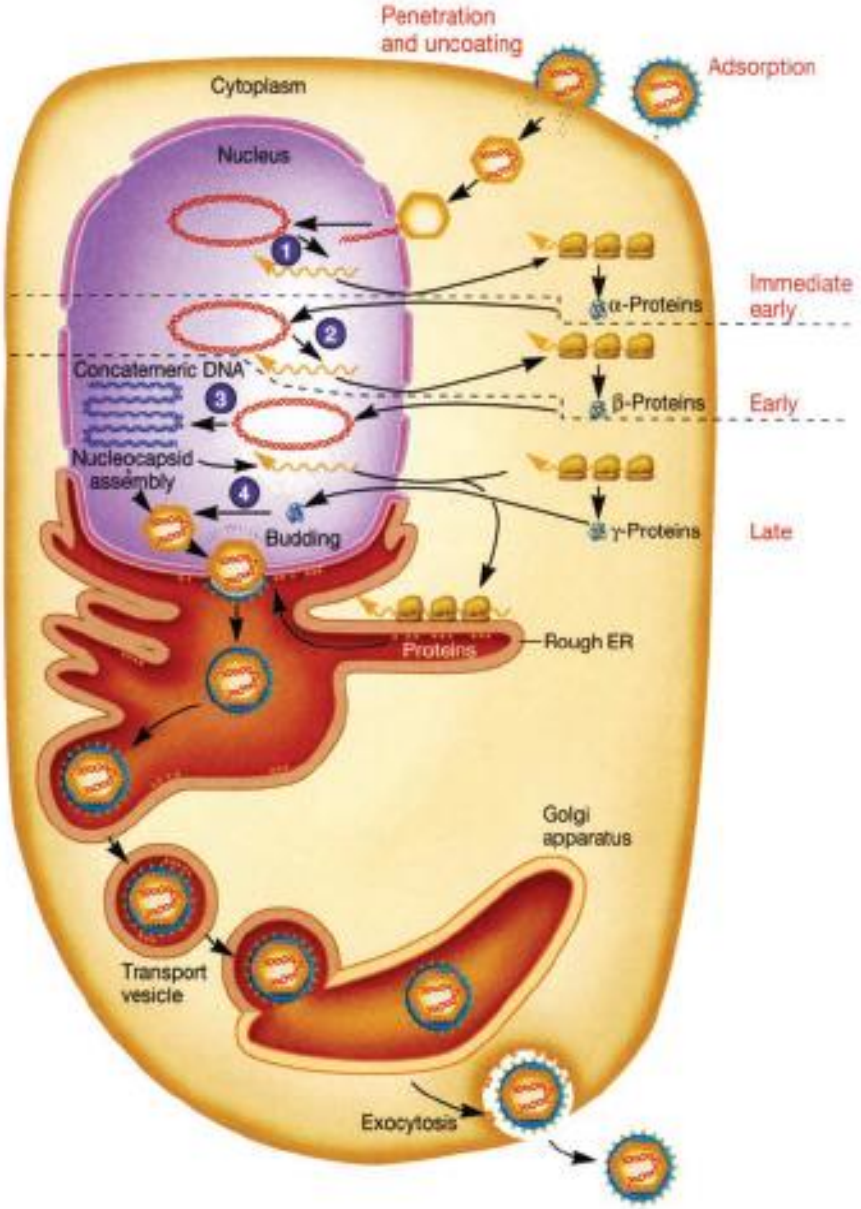
Herpetoviridae
-replication-

- The virus enters cells after the interaction of viral glycoproteins and cell-surface receptors; followed by fusion with the plasma membrane; migration to the nucleus where the genome is released, forms a circular structure and can initiate the expression of viral genes:

(1) immediate-early (IE) mRNA , α genes- regulation of viral gene expression
(2) early (E) mRNA – viral replication
(3) late (L) mRNA – structural proteins

- Virions are assembled in the nucleus, obtain the envelope from the inner layer of the nuclear membrane and are transported through the endoplasmic reticulum and the Golgi complex when they lose and regain their envelope

- Both α and γ herpesviruses block protein synthesis in host cells by shredding mRNA resulting in death of infected cells



Herpetoviridae
-latency-

Herpesviruses usually cause:

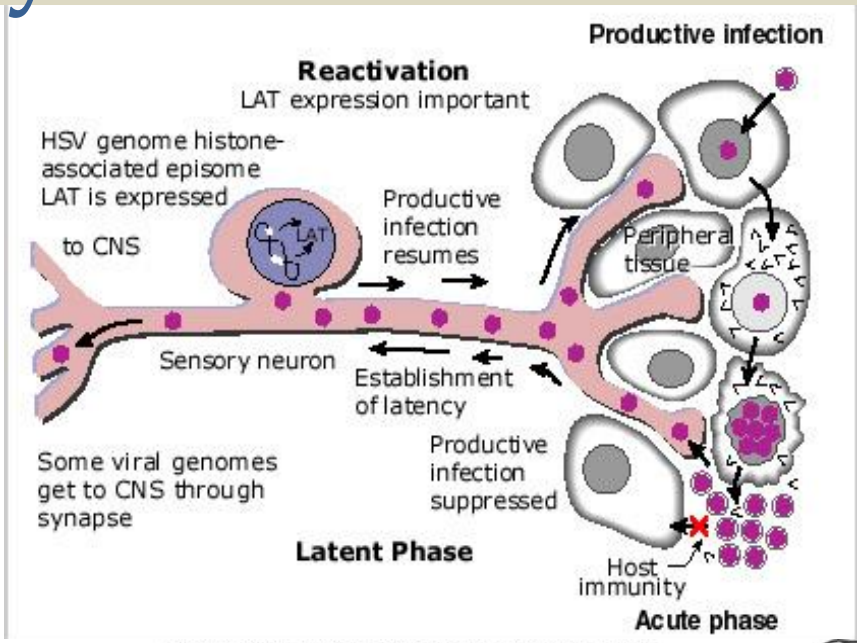
1. **initial lytic infection**, followed by
2. **latent, lifelong infection**

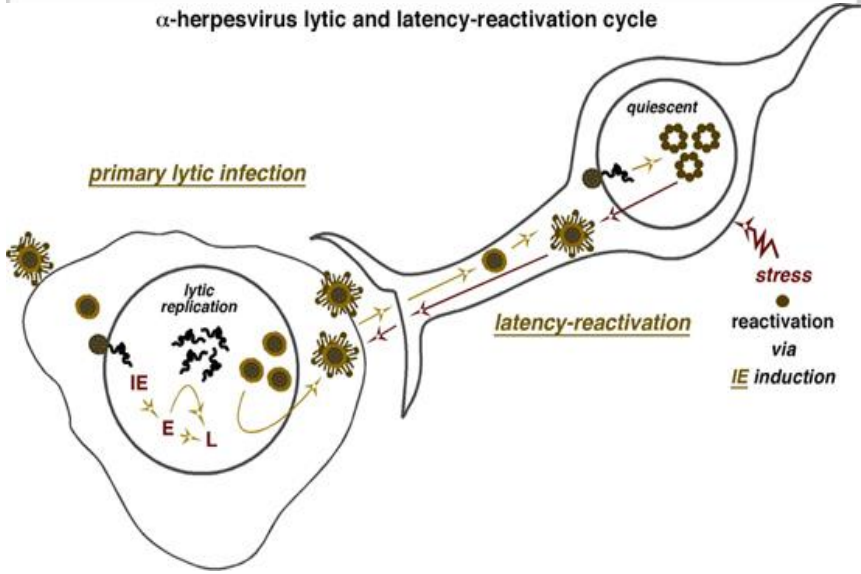
Latent infection: the virus is present in cells, but there's no replication nor forming the new virions:

- viral DNA persists as an episome in the nucleus, integration in the host chromosomes is extremely rare

- **during latency there's only minimal transcription of viral genes** (enabling the functioning and maintenance of the viral episome, preventing the death of host cells and inhibiting host immune responses)

Periodic reactivations enable a constant source of new infections in the population



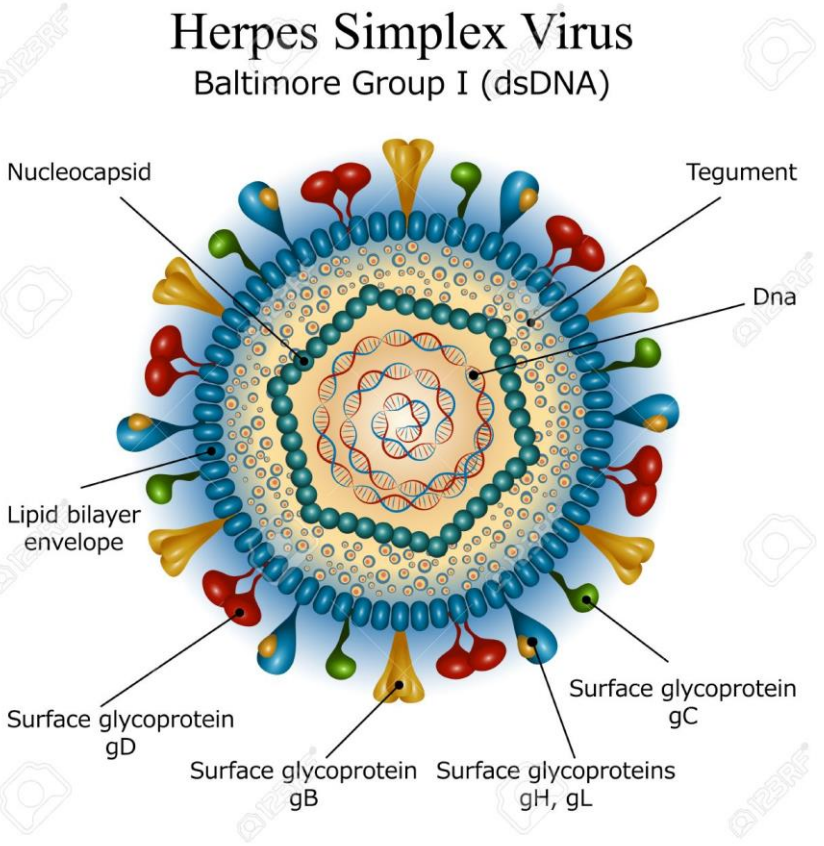


*micro*RNA - play a role in maintaining latency

Herpes simplex virus (HSV)
HSV-1 and HSV-2

- These viruses differ epidemiologically and in their antigens, but their genomes contain about 50% homology

- It is possible to distinguish these two types of viruses based on the expression of glycoprotein B and PCR analysis



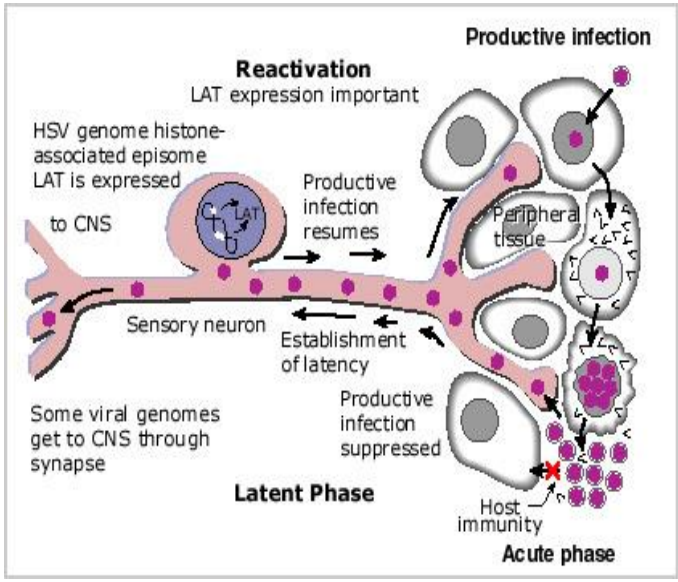
HSV-1, HSV-2
-epidemiology-

HSV-1 causes **facial herpes**, and **HSV-2** usually causes **genital infections**

- They are acquired by direct contact
- HSV-1 are HSV-2 distributed worldwide
- In developing countries, 90% of the population have anti-HSV-1 antibodies by the age of 30, and 15-30% of adults in Western industrialized countries have antibodies to HSV-2, while detecting antibodies to HSV-2 before puberty is very rare
- HSV-2 can be isolated from the cervix and urethra of 5-12% of adults (asymptomatic excretion of HSV-2 is possible)

HSV-1 and HSV-2
-pathogenesis of the infection-

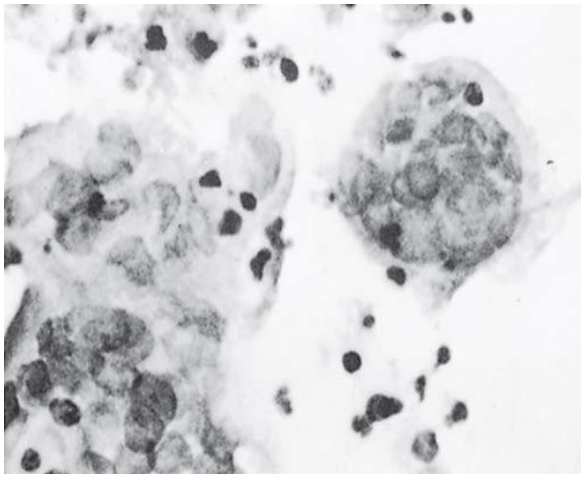
HSV-1 and HSV-2 replicate at the entry site, **in skin or mucous membranes – lytic or productive infection**



Pathological changes in acute infection:

- ✓ formation of multinucleated giant cells, epithelial cell degeneration, focal necrosis, formation of eosinophilic multinuclear inclusion bodies
- ✓ inflammatory response: polymorphonuclear and mononuclear cell infiltrate
- ✓ the virus spreads to local sensory neurons and ganglia that innervate the area of infection

Latency is established in neurons of the ganglia



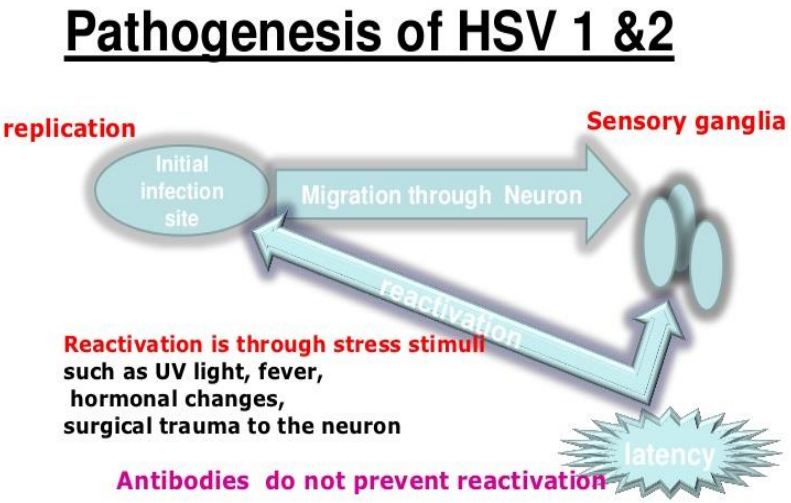
HSV-1 and HSV-2
-pathogenesis-latency-

HSV-1 latency : trigeminal ganglia, superior cervical and vagal ganglia and, rarely, in the dorsal sensory ganglia (S2-S3)

HSV-2 latency: sensory ganglia of the sacral region (S2-S3)

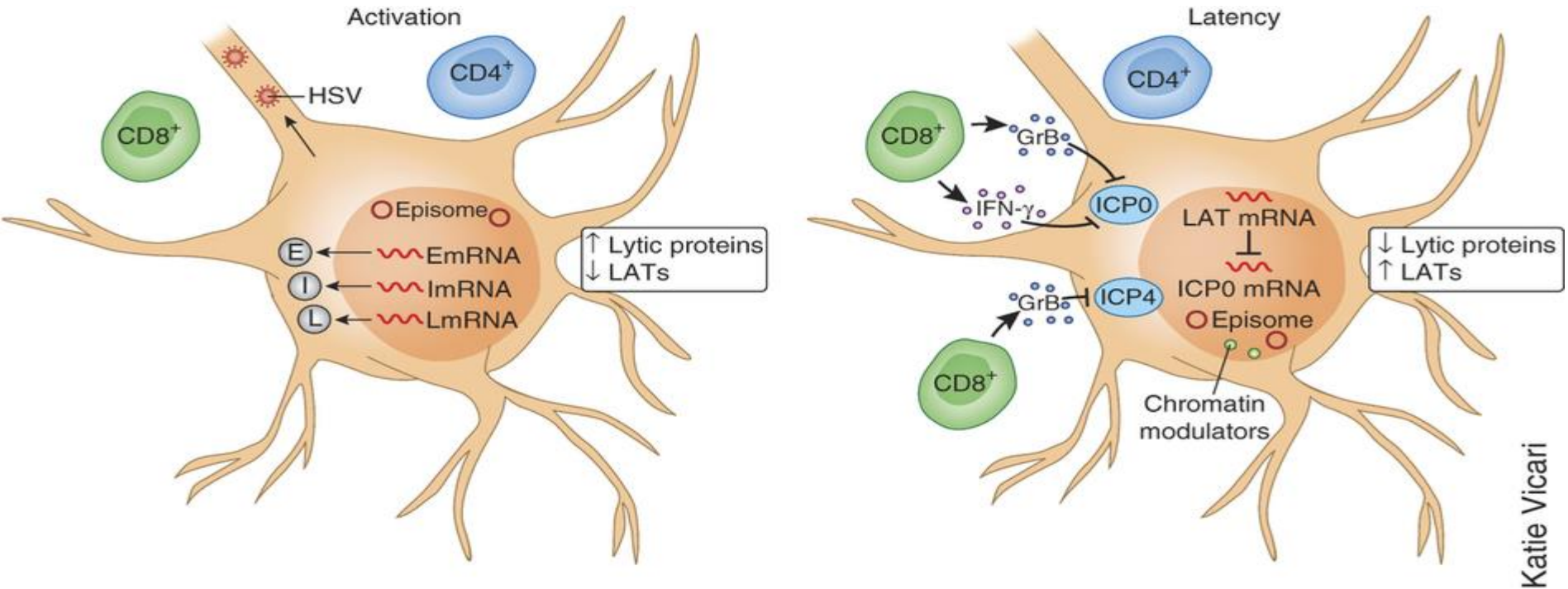
Elimination of latent infections is impossible!!!

- The virus is occasionally reactivated and causes clinical manifestations (reactivation is possible even without clinical manifestations)
- Predisposing factors preceding reactivation: UV exposure, fever, emotional stress, trauma...



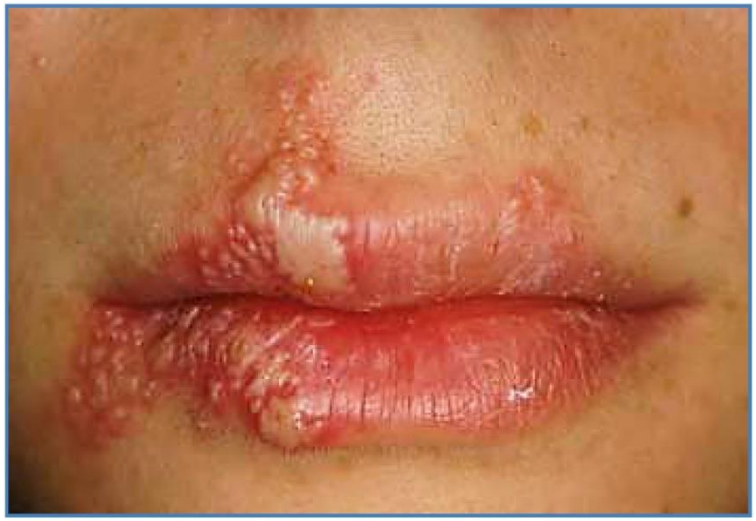
HSV-1 and HSV-2
-immunity: neutralizing antibodies and cellular immunity-

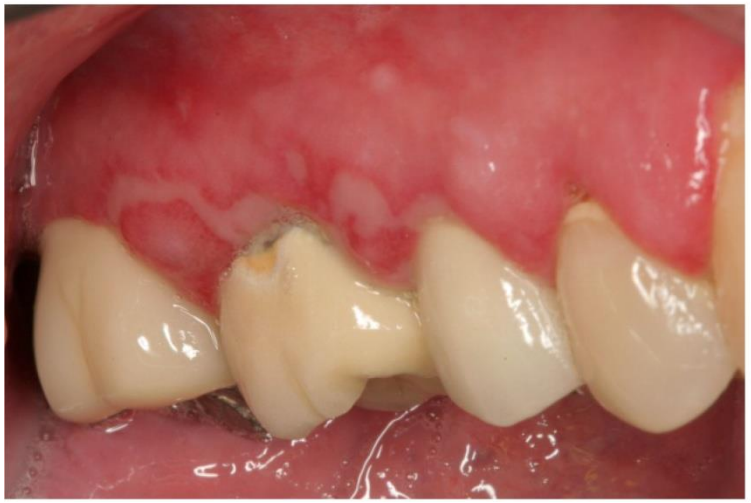
- In individuals with cellular immune deficiency, HSV reactivation may be associated with prolonged virus excretion and persistence of lesions
- **Circulating antibodies** protect against exogenous infections, and **cytotoxic lymphocytes** control the spread of the virus
- **During latency, HSV-1 and HSV-2** do not express viral proteins, so **they hide well from the immune system**
- However, reactivation is much more common in immunodeficient individuals



HSV-1
-clinical manifestations-

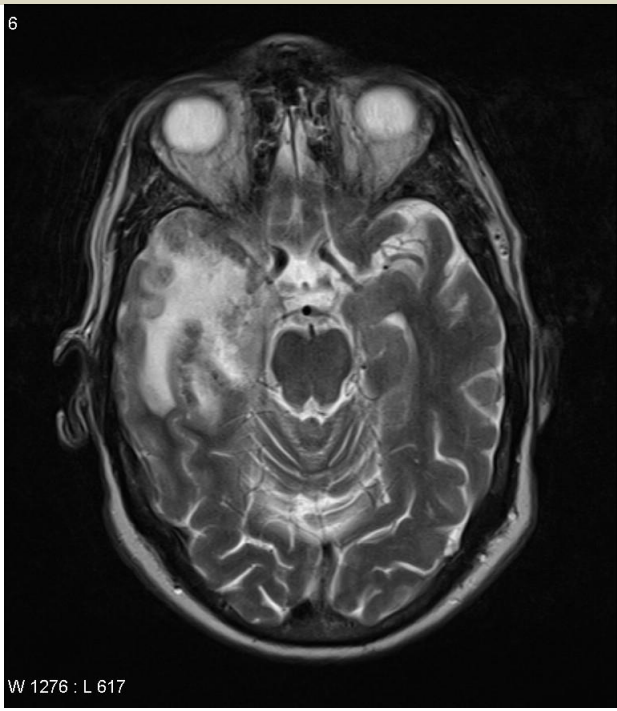
- **Ectoderm infection** (skin, mouth, conjunctiva and nervous system)
- Clinically, HSV-1 infection is characterized by the appearance of grouped or individual **vesicles** that can become **pustules**, coalesce, and later burst and give multiple and single **ulcerations**.
- HSV-1 can generally be isolated from all of these lesions, but the virus titer decreases as the lesion progresses.



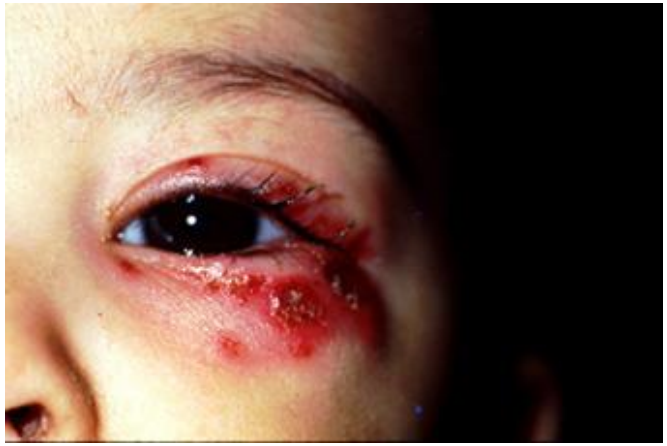


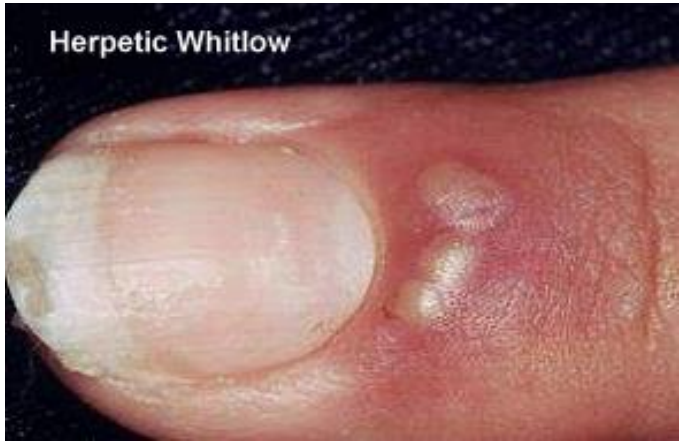
HSV-1
- clinical manifestations -

Primary herpes:
- Gingivostomatitis, most common
- Conjunctivitis and keratitis
- Encephalitis
- Herpetic whitlow (around nails)



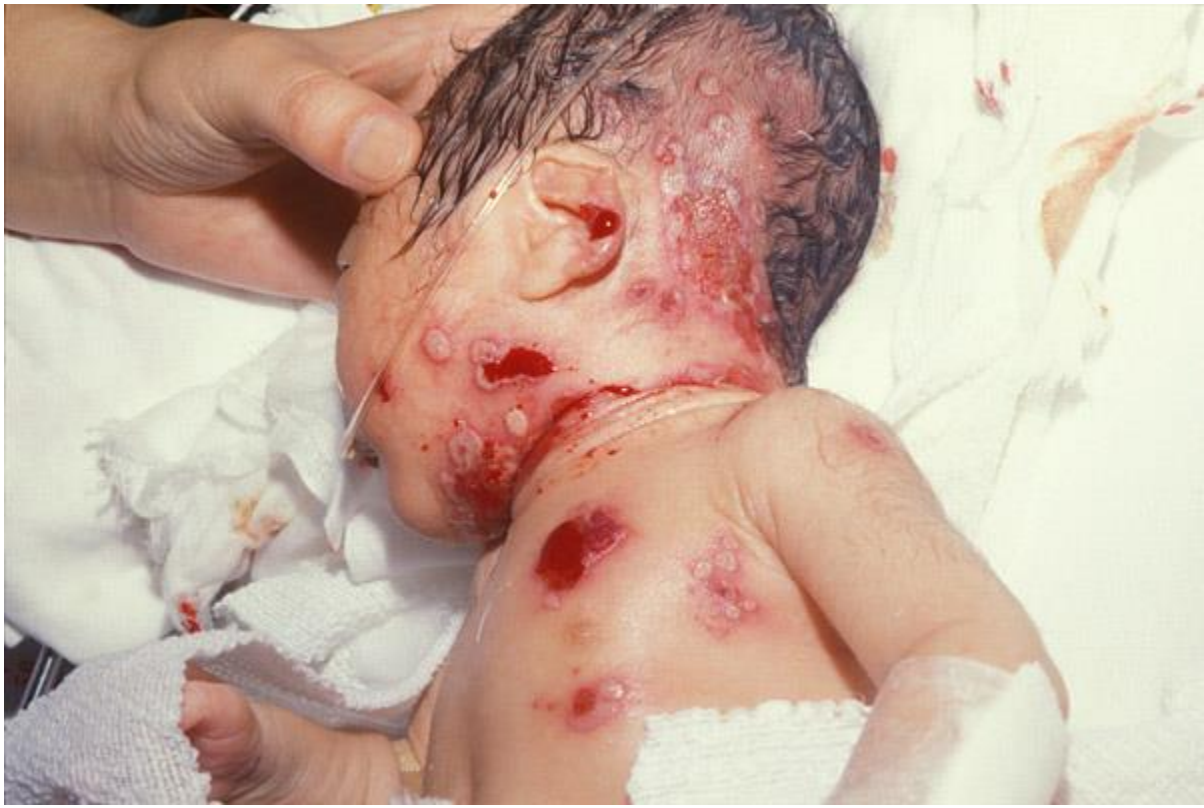
Virus reactivation:
- Cold sores
- Encephalitis





HSV-2
- clinical manifestations -

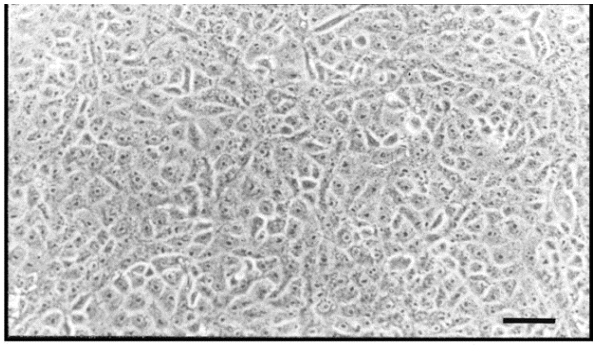
- Primary genital herpes
- Recurrent genital herpes
- Neonatal herpes

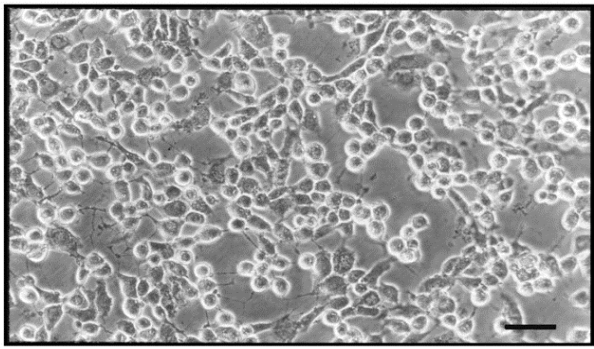


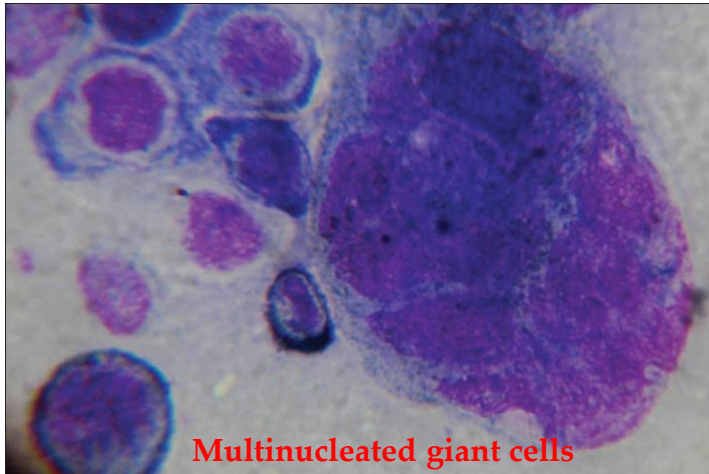
HSV-1 and HSV-2
-diagnosis-

- Virus isolated from infected secretions or lesions gives a **cytopathic effect** in culture **in 24 to 48 hours**
- HSV-1 and HSV-2 can be **stained directly with specific** fluorescein-labeled **antibodies**
- **Direct preparation**, *Tzanck* test (intranuclear inclusions in multinucleated giant cells)
- **PCR** of blood and cerebrospinal fluid samples
- Serological reactions have no diagnostic significance, except for the detection of asymptomatic genital herpes

Cytopathic effect in culture





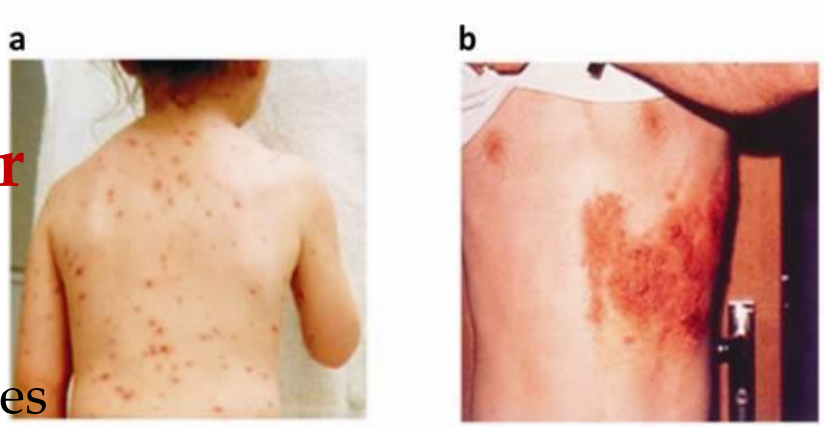


Multinucleated giant cells

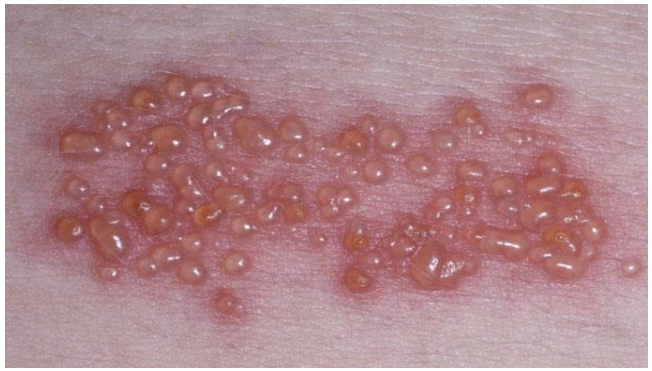
Varicella-zoster virus (VZV)

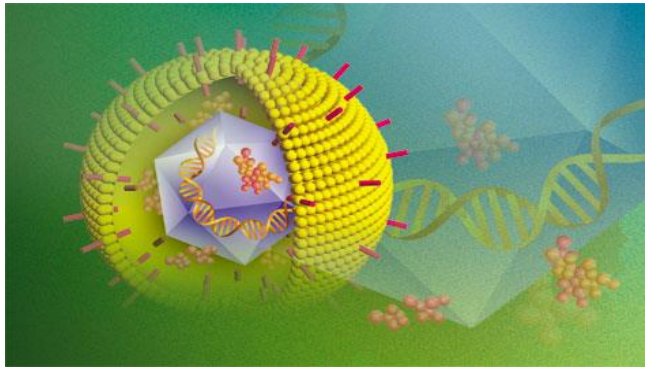
VZV causes two diseases:

***varicella* (chickenpox)** and **zoster (shingles)**



- The smallest genome among herpesviruses
- VZV codes thymidine kinase – sensitive to acyclovir
- VZV is more difficult to propagate in cell culture than HSV, because VZV generally remains bound to the host cell membrane with less release of virions in the fluid, it spreads poorly from cell to cell. It does not grow well in culture





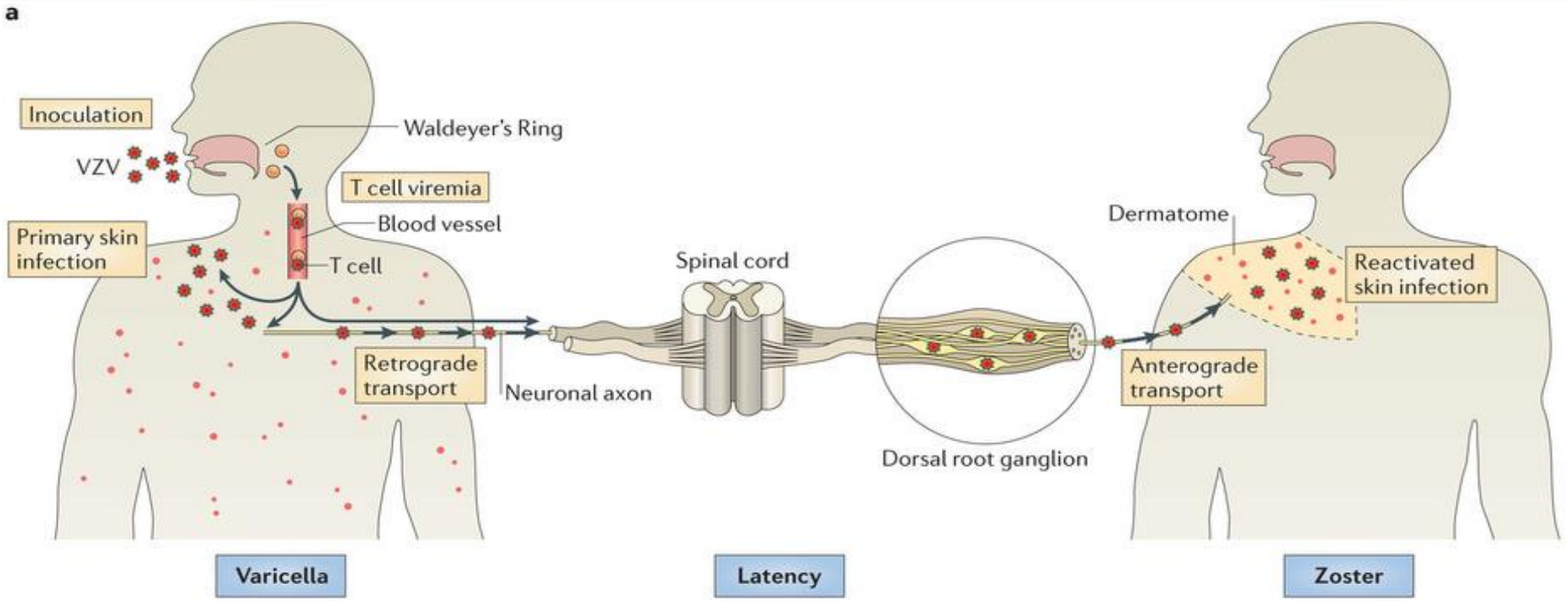
VZV
- epidemiology-

- VZV is ubiquitous
- In temperate climates, more than 90% of people come into contact with this virus in childhood, and the disease occurs by the age of ten.
- The disease occurs in 75% of people who have been in contact with the virus - **the most contagious herpes virus**
- **Shingles** is caused by reactivation of VZV and occurs in 20% of the population (mostly after the age of 50)

- The most common **route of transmission** of the virus is **respiratory,** although transmission by **direct contact with vesicles and pustules** is also possible
- The transmission of the disease is greatest 1 to 2 days before and 3-4 days after the appearance of skin lesions
- The virus is difficult to isolate from the lesions that enter the crust phase

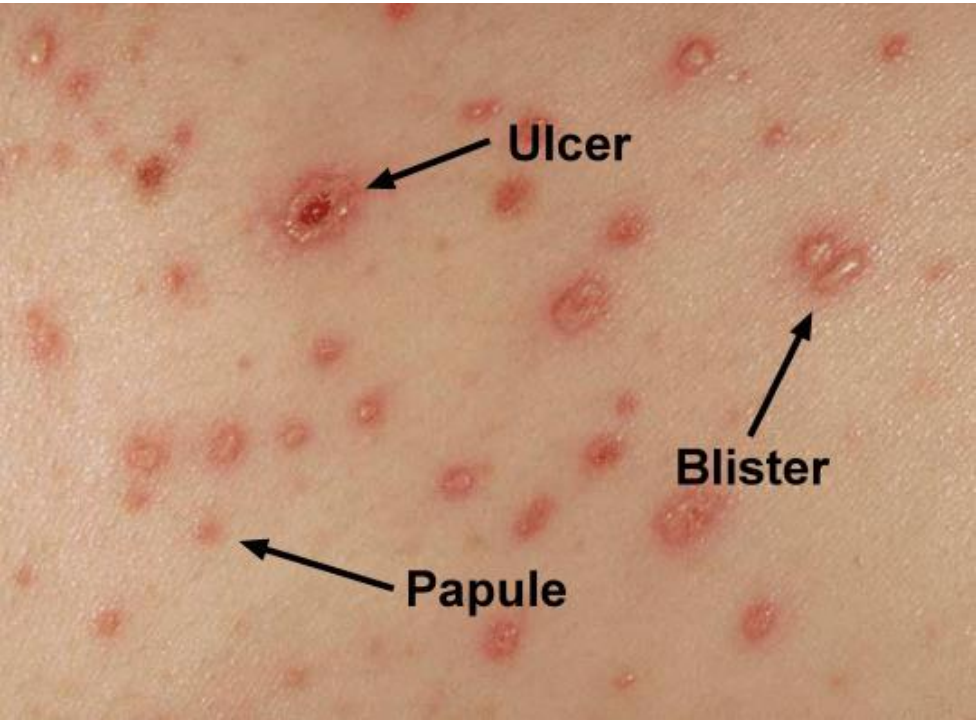
VZV
- pathogenesis, immunity-

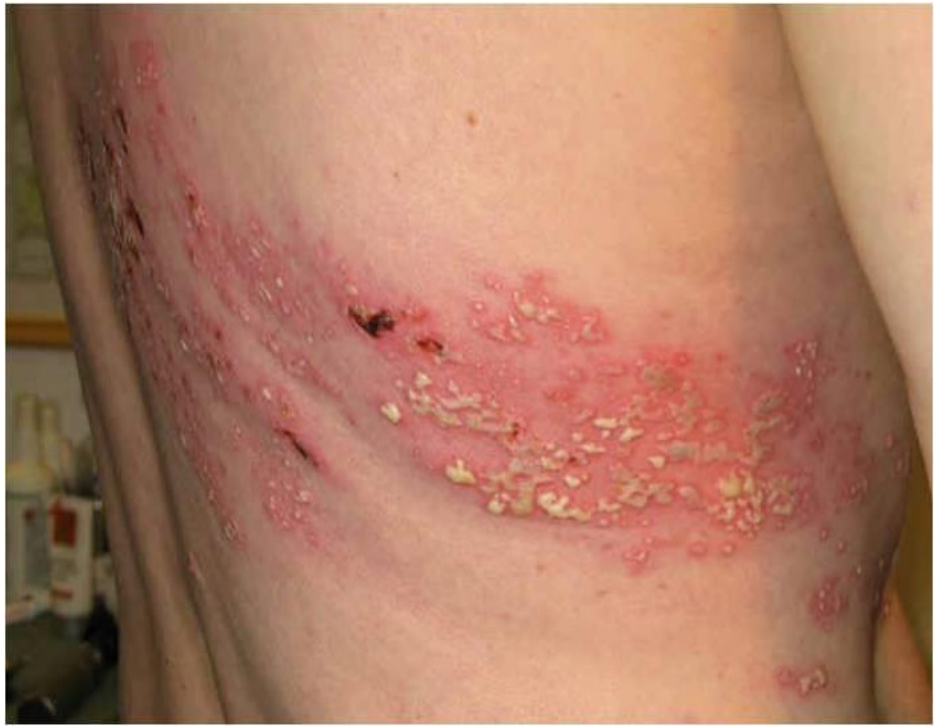
- Primary and secondary viremia
- The virus spreads through cells (T lymphocytes and monocytes), **cellular immune response is key to control the infection**
- Establishes **latency in sensory trigeminal ganglia and dorsal spinal ganglia**
- Reinfection with this virus is rare and is prevented by circulating antibodies, while reactivation is mainly controlled by cellular immunity



VZV
- clinical manifestations -

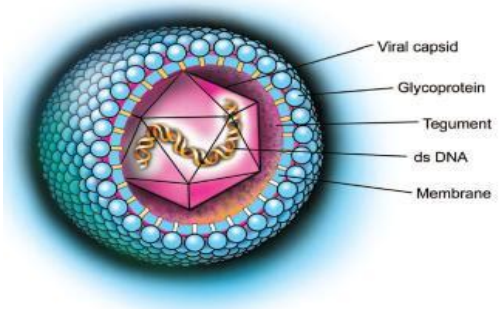
- ***Varicella* or chickenpox: generalized skin rash**, in one area lesions can be observed in different stages (from vesicle to crust)
- In case of **immunodeficiency**, **prolonged viremia** and virus **dissemination** (pneumonia, encephalitis, hepatitis and nephritis) occur.
- **Reactivation of** VZV is associated with **zoster (shingles)**
- Herpetic neuralgia



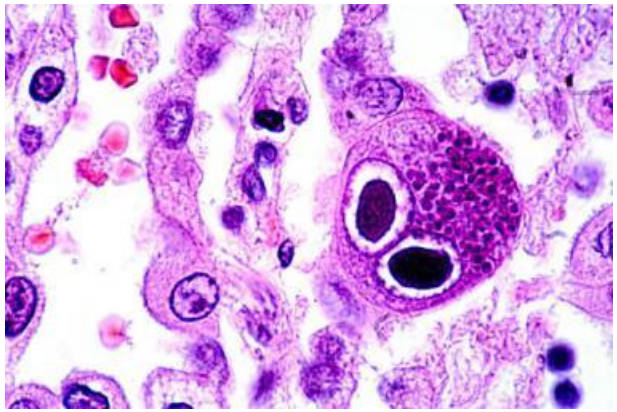


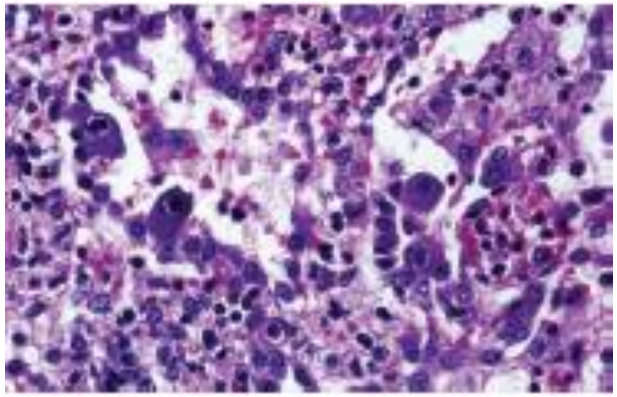
Cytomegalovirus (CMV)

- CMV causes **mononucleosis syndrome** in immunocompetent individuals; **congenital infections** (1% of all newborns) and **severe disease and death in immunodeficient individuals**



HCMV Human Cytomegalovirus





- It is named after the pathogenic effect it causes in cell culture (nuclear and perinuclear inclusions and cell enlargement - cytomegaly)
- It belongs to β subfamily of herpesviruses
- CMV has the largest genome of all herpesviruses
- Based on genetic and phenotypic heterogeneity, several strains of CMV have been identified

Cytomegalovirus (CMV) -epidemiology-

CMV is ubiquitous

In developed countries, about 50% to 70% of adults have antibodies to CMV, and the percentage is significantly higher in poorer socioeconomic conditions.

Mode of transmission:

- The virus is usually transmitted by **close contact**, including sexual intercourse **with a person who excretes the virus** (CMV can be isolated from saliva, cervical secretions, semen, urine and leukocytes months and years after infection)
- Transfusion (rarely)
- Organ transplant

CMV
- pathogenesis, immunity-

- CMV **infects vascular endothelial cells and leukocytes**, in which it forms characteristic inclusions
- CMV causes disease by various mechanisms, including direct tissue damage as well as damage by the host's immune response (humoral and cellular immunity)
- **In its latent form, it is present in monocytes and monocyte precursors** ($CD34^+$ cells)
- CMV infection of monocytes results in dysfunction of these cells in immunodeficient individuals, which may increase the predisposition to develop fungal and bacterial superinfections
- Virus reactivation and excretion by cervical excreta is mostly subclinical

CMV
- clinical manifestations -

Congenital infection:

- 1% of newborns
- **Asymptomatic congenital infection** (90% of cases), 10-20% later develop sensory deafness, psychomotor mental retardation or both
- **Symptomatic disease** (around 0,1% of all childbirths): various congenital disorders, hepatosplenomegaly, jaundice, anemia, thrombocytopenia, low birth weight, microcephaly, chorioretinitis
- Almost all children with clinically evident congenital CMV infection were born to mothers who had a primary CMV infection during pregnancy

Primary infection in young adults – mononucleosis syndrome

Infection of immunodeficient people – severe pneumonia, chorioretinitis, gastroenteritis, neurological disorders

Recommended procedures for proving CMV infection in certain clinical conditions:

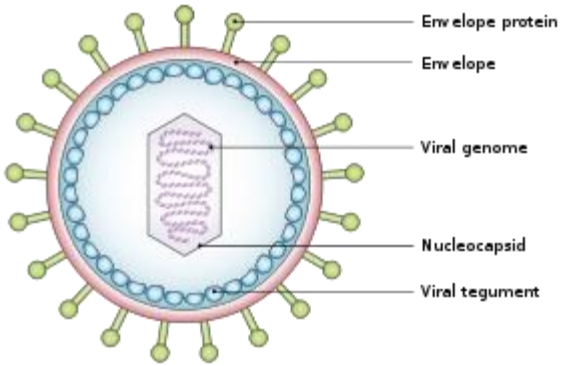
1. **Congenital infection** – virus culture or detecting viral DNA at birth or within 1 to 2 weeks (perinatally infected children do not excrete the virus 3 to 4 weeks after birth)

2. **Perinatal infection** - negative cultures at birth and positive 4 or more weeks after birth indicate natal or early postnatal infection. Seronegative newborns can acquire CMV from external sources such as blood transfusions

3. **CMV mononucleosis in immunocompetent people** - The presence of CMV-specific IgM antibodies is the best indicator of primary infection. A positive urine culture can indicate a CMV viral infection, but it can also indicate a previous infection because the disease can last for months and years. Positive finding of CMV antigen or viral DNA in the blood

4. **Immunodeficient people** - detection of viral antigens or DNA in the blood indicates viremia. Demonstration of inclusions or viral antigens in damaged organs indicates the presence of CMV infection, but does not prove that CMV is the cause of the disease until other pathogens are ruled out. CMV-specific IgM antibodies do not have to be present in immunodeficient patients. In AIDS patients, CMV-specific antibodies are generally present even when there is no clinically manifest disease

Epstein-Barr virus (EBV)



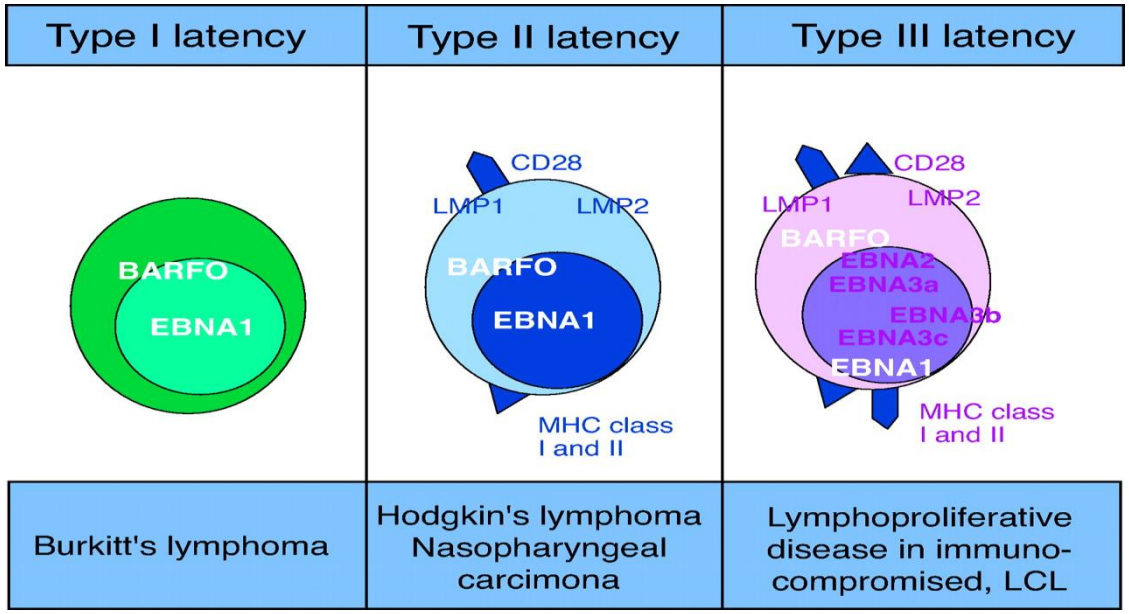
EBV is the causative agent of:

1. **infectious mononucleosis**
2. **african Burkitt lymphoma and nasopharyngeal carcinoma**
3. **lymphoproliferative diseases in immunodeficient individuals**

- Belongs to subfamily of γ herpesviruses
- Shows tropism for human B lymphocytes and epithelial cells
- In latent form it is present in B lymphocytes

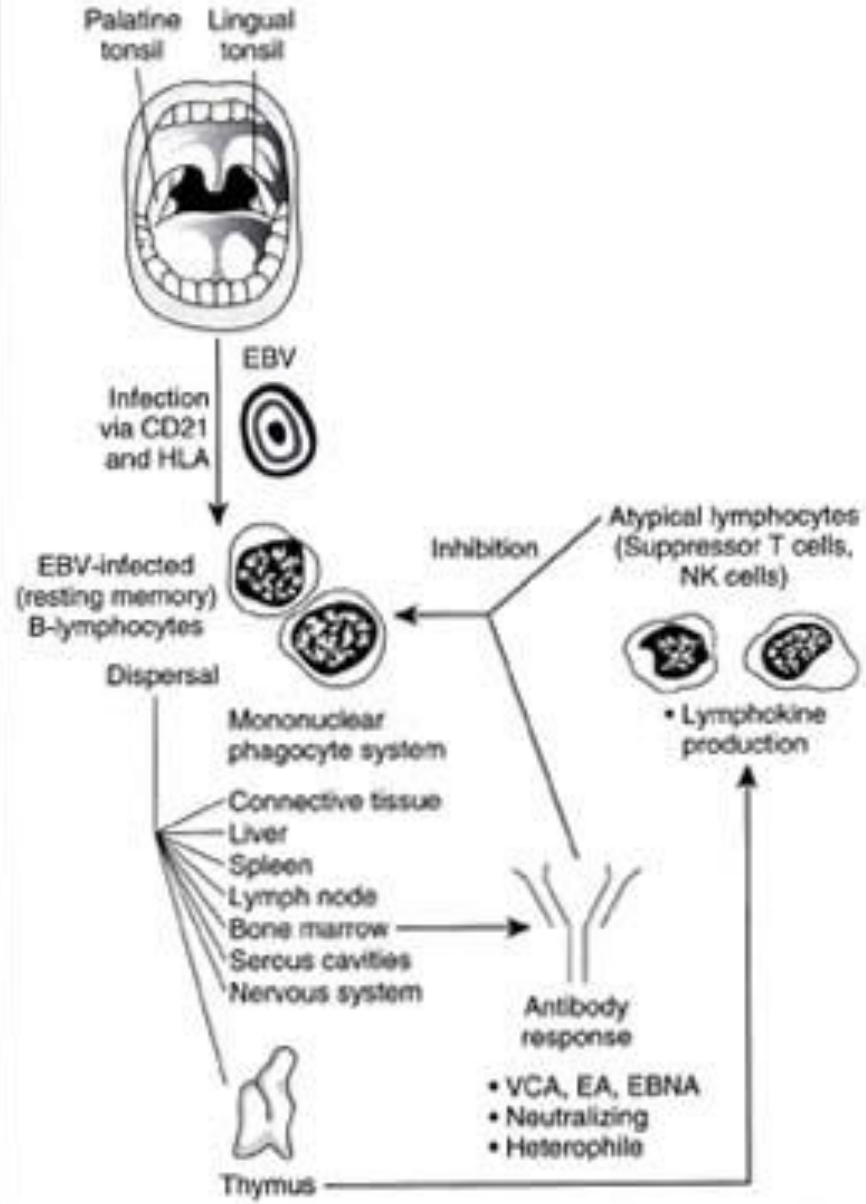
EBV
-latency-

- **type III latency (in lymphoblasts):** 4 Epstein-Barr nuclear antigens are expressed (**EBNA genes**), including EBNA-1 whose products are necessary for maintenance of viral genome in episomal form, and two integral membrane proteins, and many regulatory miRNA
- **type II latency (Hodgkin's lymphoma and nasopharyngeal carcinoma cells):** not full spectrum of EBNA proteins is expressed, but numerous other viral genes are
- **type I latency (in Burkitt's lymphoma):** expression of only EBNA-1 and regulatory miRNA



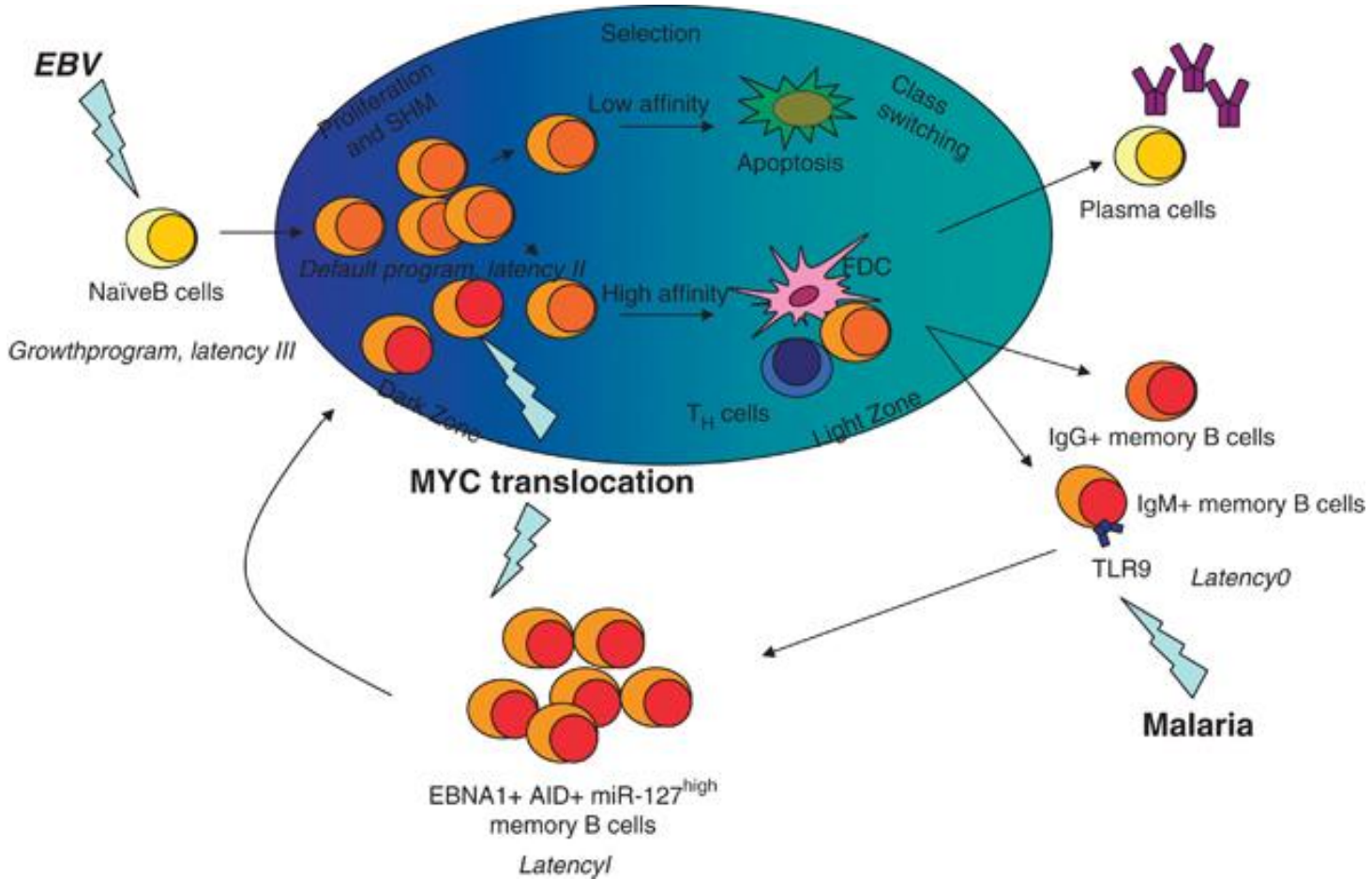
EBV
-pathogenesis-

- Initial infection of **oral epithelial cells**
- Late infection of **B lymphocytes** (through **CR2 receptor**) and polyclonal activation of B cells followed by benign proliferation of these cells
- Infected B lymphocytes express viral antigens targeted by antiviral immune responses



EBV
-pathogenesis-

- Transformation of cells: LMP-1 gene expression, c-*myc* translocation (in lymphomas)
- Cofactors in tumor formation are immunosuppression, malaria



EBV
- epidemiology, immunity-

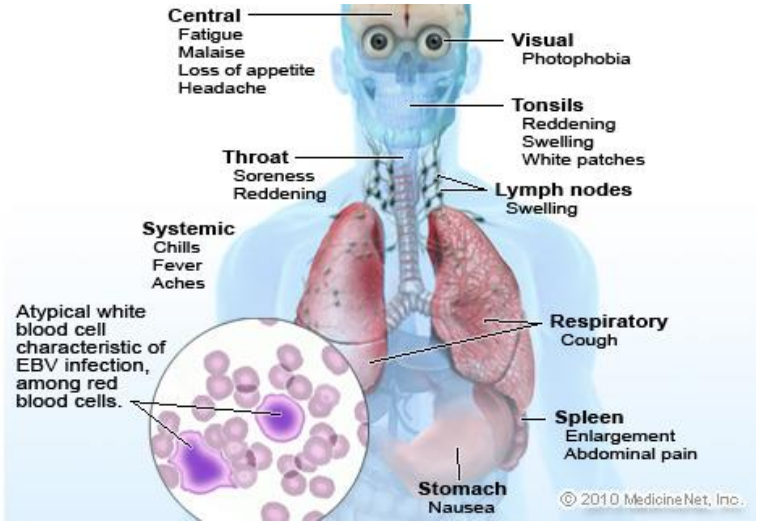
- Over 90% of the population have EBV-specific antibodies
- In developing countries, most children become infected before the age of 2, while in developed countries the infection is more common in later childhood and adolescence
- When EBV infection occurs in the second decade of life or later, it is associated with infectious mononucleosis in about 50% of cases
- EBV spreads through **direct contact with oropharyngeal secretions** (can be isolated from the saliva of 10-20% of healthy adults and seropositive individuals)
- It is less contagious
- Infectious mononucleosis can also be transmitted by blood transfusion, although in this case it is mainly caused by CMV

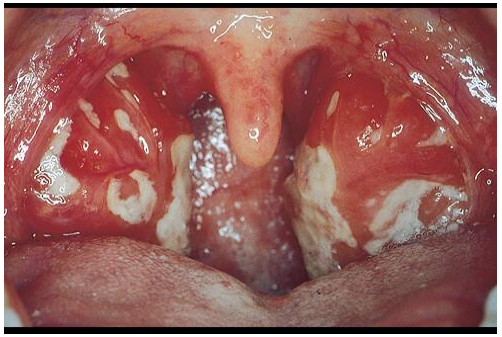
As a response to viral presence a **strong cell-mediated immune response** develops, and **atypical lymphocytosis** occurs (activated $CD8^+$ T lymphocytes)

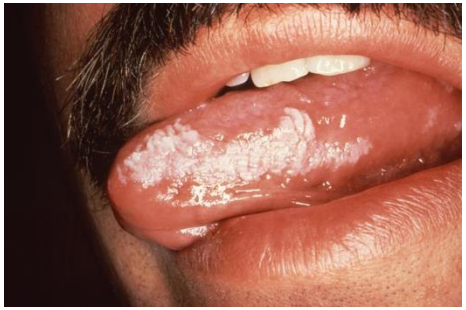
Weakened cellular immune response is detected early in the course of the disease

EBV
-clinical manifestations-

- **Infectious mononucleosis :** (fever, fatigue, pharyngitis, painful lymphadenitis, splenomegaly)
- **Lymphoproliferative syndrome:** (in states of immunosuppression) persistent fever, lymphadenopathy and hepatosplenomegaly
- **Burkitt lymphoma**
- **Nasopharyngeal carcinoma**
- **Tongue leukoplakia, lymphocytic interstitial pneumonia** in **AIDS** patients





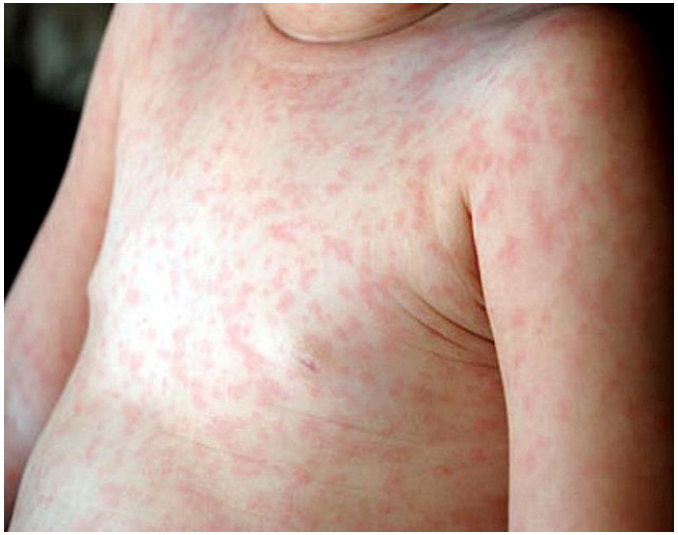


HHV-6

- Belongs to the subfamily of β herpesviruses
- **Ubiquitous**, by the age of five everyone has anti-HHV-6 antibodies
- **It replicates in lymphatic tissue**, especially **CD4⁺ T lymphocytes**
- Causes **roseola**, but also **an acute febrile state without the rash**
- **It reactivates in immunosuppressed individuals** and contributes to the development of severe pneumonias, encephalitis...

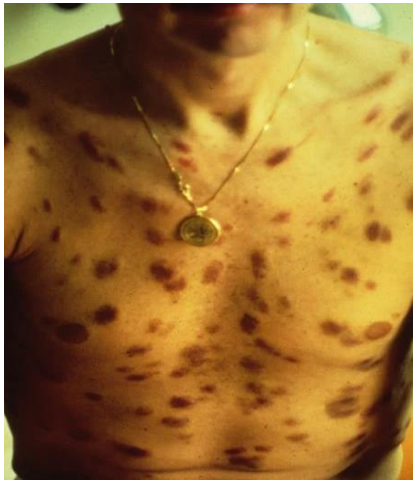
HHV-7

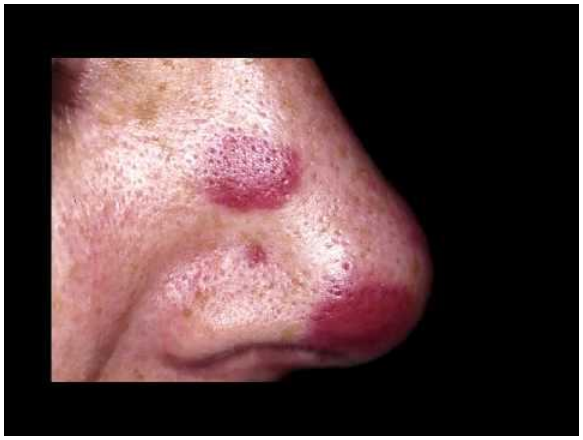
- Around 90% of children have antibodies to this virus by the age of three
- It may cause **roseola (*exanthema subitum*)** which is confirmed only in rare cases

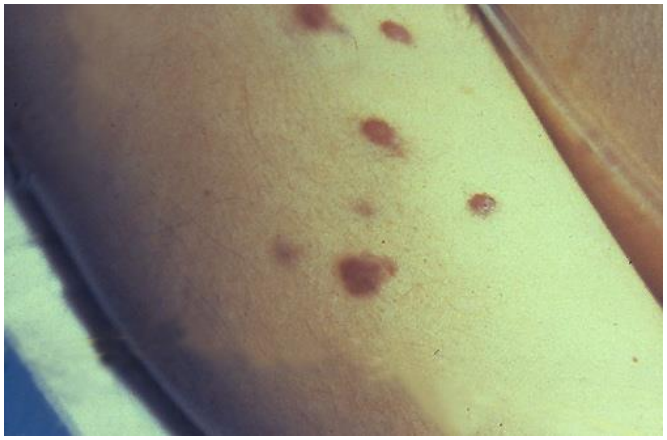


HHV-8

is involved in the development of Kaposi sarcoma







- The seroprevalence of HHV-8 can reach 50%, and in some areas (Central Africa) Kaposi sarcoma is endemic
- Prolonged direct contact is required for virus transmission
- HHV-8 infects the epithelium of the oral mucosa and is secreted with saliva into the external environment. It can also be found in B lymphocytes of the peripheral blood
- It is present in tumor cells (spindle cells of endothelial origin), mostly in a latent state, and only occasionally expresses lytic genes

HHV-8
-clinical manifestations-

1. **Classic Kaposi sarcoma** was described by Moritz Kaposi in the 1800s. It is a rare, indolent tumor that mainly occurs in the lower extremities of older men in the Mediterranean, and has also been described in Ashkenazi Jews.
2. **Endemic Kaposi sarcoma**, common in Central Africa, more aggressive than classic, affects mainly the upper extremities, oral mucosa and torso
3. **Iatrogenic Kaposi sarcoma** – occurs after transplantation, but resolves after discontinuation of immunosuppressive drugs
4. **Epidemic Kaposi sarcoma associated with AIDS** is the most destructive form of Kaposi sarcoma, tumors appear in the mouth, torso, face and can be found in internal organs. Without HIV treatment, it is deadly

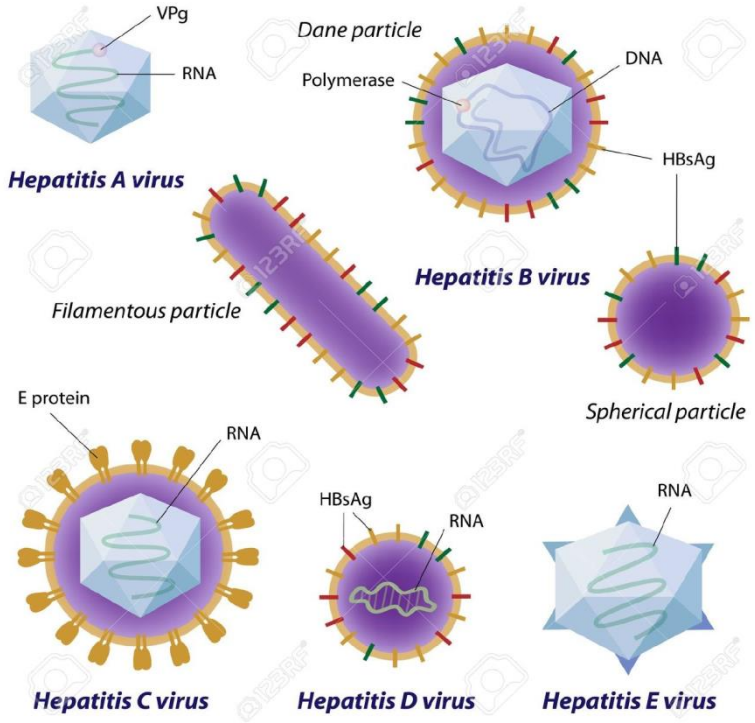
Primary effusion lymphoma has a higher mortality rate compared to Kaposi sarcoma and is 100% associated with HHV-8. EBV is present in 50 to 70% of cases of primary effusion lymphoma and may play an additional role in the development of the disease. There are no known genetic abnormalities in patients with this disease

Viral hepatitis

Viral hepatitis is a clinical syndrome characterized by fever and signs and symptoms of liver damage

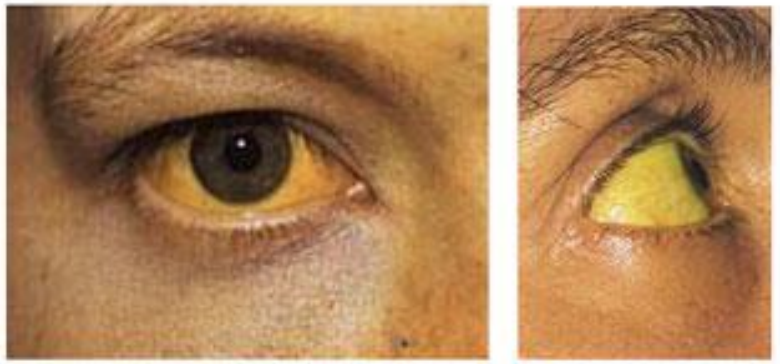
Many viruses (adenoviruses, CMV, EBV, HSV, VZV and yellow fever virus) may infect liver and cause the disease. However, all these viruses can also cause extrahepatic disease

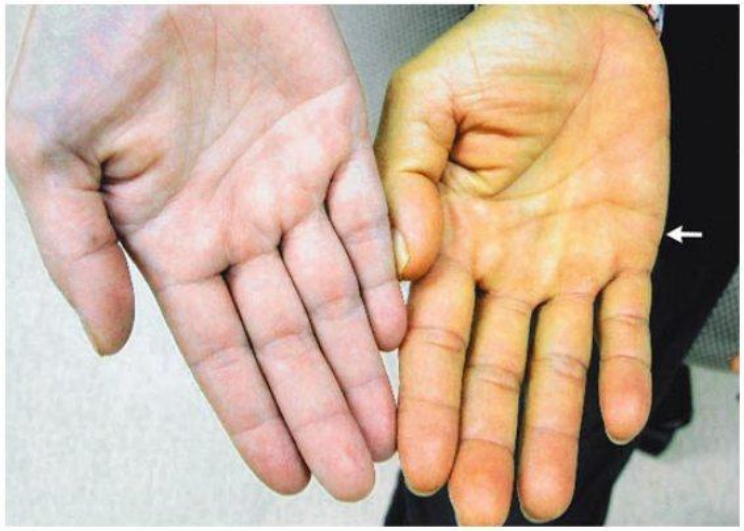
"Professional" hepatitis viruses have a strong tropism for the liver, mainly replicate in hepatocytes and rarely cause extrahepatic diseases.



Viral hepatitis
-clinical manifestations-

- **Fever, abdominal pain, nausea and vomiting** are common in mild cases
- As liver damage becomes more severe, bilirubin accumulates in the skin, causing a **yellowish discoloration known as jaundice**
- In even more severe cases, **mental function** is impaired due to the inability to detoxify toxins absorbed from the gastrointestinal tract and **bleeding** due to reduced production of coagulation factors





Laboratory tests show elevated serum bilirubin and transaminases from damaged hepatocytes

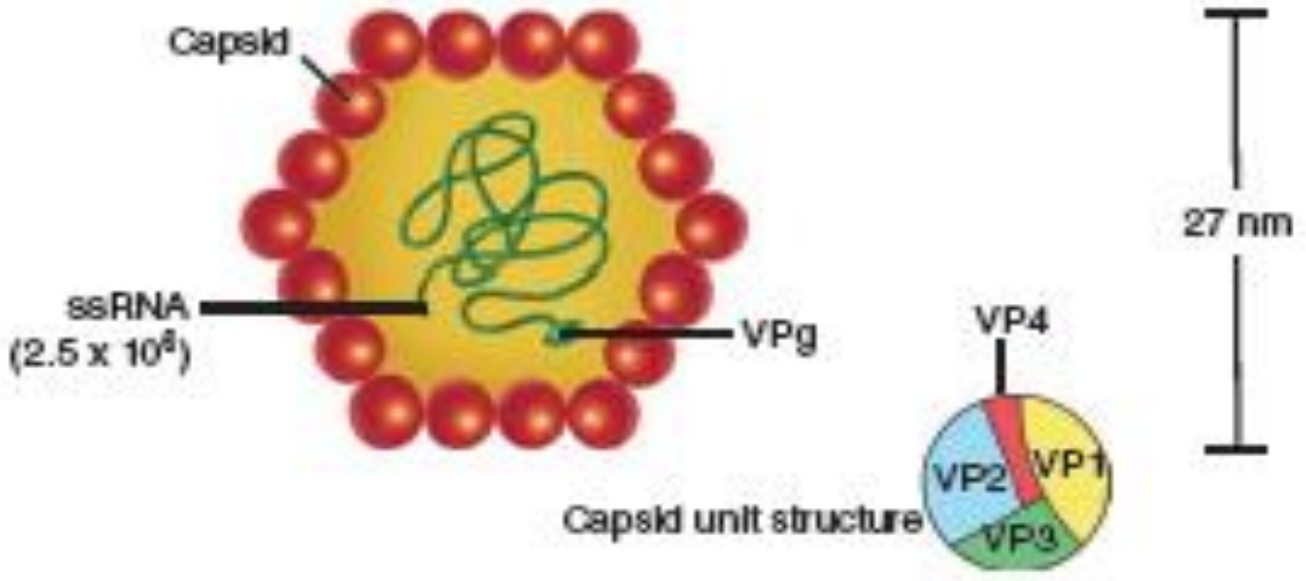
Hepatitis viruses

Comparative characteristics of hepatitis A, B, C, D and E viruses

Feature	A	B	D	C	E
Virus type	Single-stranded RNA	Double-stranded DNA	Single-stranded RNA	RNA	RNA
Incubation (days)	15-45 (average 25)	30-180 (average 60-90)	28-45	15-150 (average 50)	21-56 (average 40)
Beginning	Usually sudden	Gradual	Variable	Insidious	?
Age of most common occurrence	Children, young people	All ages	All ages	All ages	Younger people
Fecal-oral transmission	+++	±	±	-	+++
Sexual transmission	+	++	++	+	+?
Parenteral transmission	-	+++	++	+++	
Chronic infection (%)	No	10	50-70	85	Rarely
Carriers	No	Yes	Yes	No	No
Protective serum antibodies	Yes	Yes	Yes	No	No
Vaccine	Yes	Yes	Yes	No	No

Hepatitis A virus (HAV)

- HAV belongs to *Picornaviridae* family and genus *Hepatovirus*, is nonenveloped virus with icosahedral symmetry
- HAV genome is made of single-stranded RNA to which the VPg protein is bound, and each capsid unit contains 4 proteins VP1, -2, -3 и -4
- VP1 binds to a receptor on host cells
- There is only one serotype of HAV virus



HAV
- epidemiology-

- **The infection is subclinical in 50% of infected adults**, and when it is symptomatic, it is usually manifested by fever and jaundice, **chronic hepatitis is very rare**

- The most important source of HAV are humans, it is **transmitted feco-orally**, transmission by transfusion is possible, but rather uncommon

- Carrier state is possible, but uncommon

- Mostly sporadic cases occur, epidemics are rare

- The person is most contagious during the two weeks before the onset of clinical manifestations of the disease

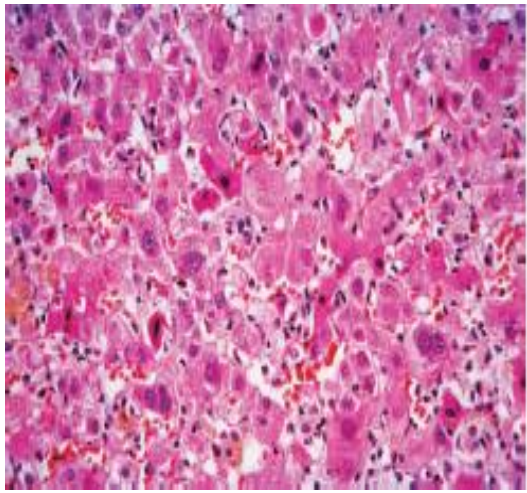
HAV
- pathogenesis -

1. **Initial replication in the intestinal mucosa** (with the onset of symptoms, the virus can no longer be found in the feces)

2. **Viremia with spread of the virus to the liver**

3. **Viral replication in liver and inflammation** – Virus and cytotoxic T cells damage hepatocytes

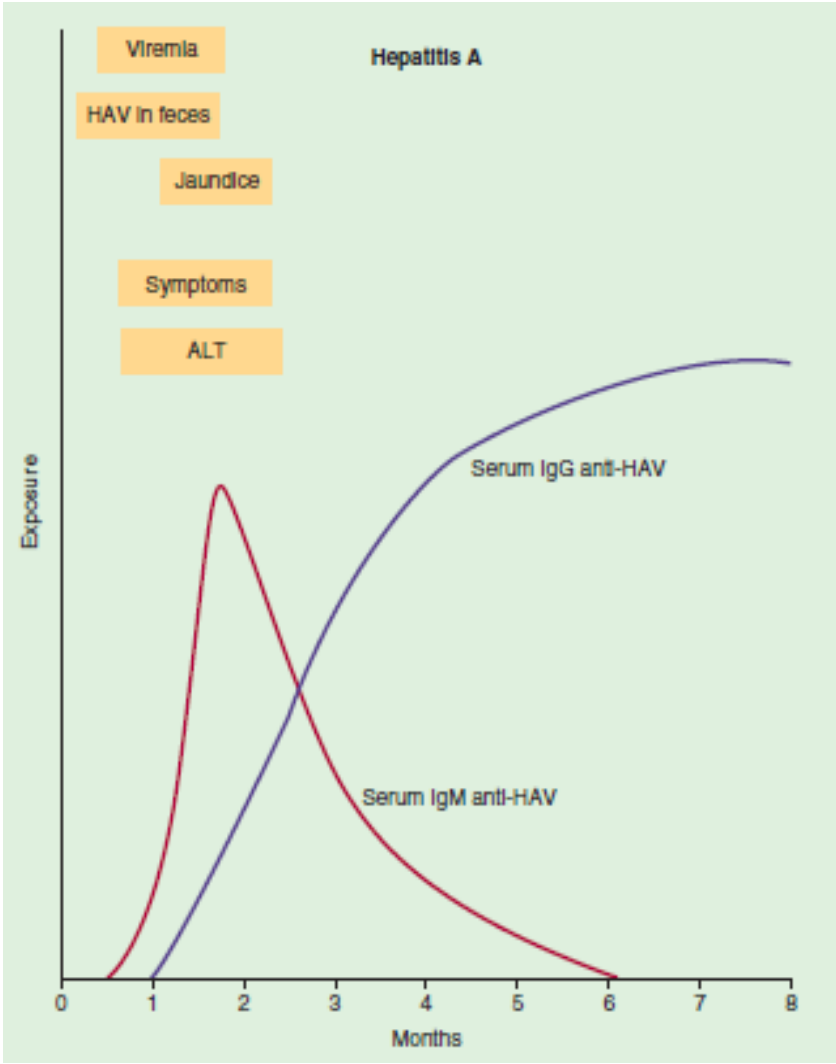
4. **Elimination of infection** (liver damage is reversible)



The initial immune response to infection involves the production of HAV-specific **IgM** antibodies, followed by **IgG** in a few weeks. Detected levels of antibodies to HAV permanently persist in serum, so people who have anti-HAV antibodies are immune to reinfection. Although virus-specific IgA antibodies have been detected in the stool, mucosal immunity is not important in protecting against hepatitis A

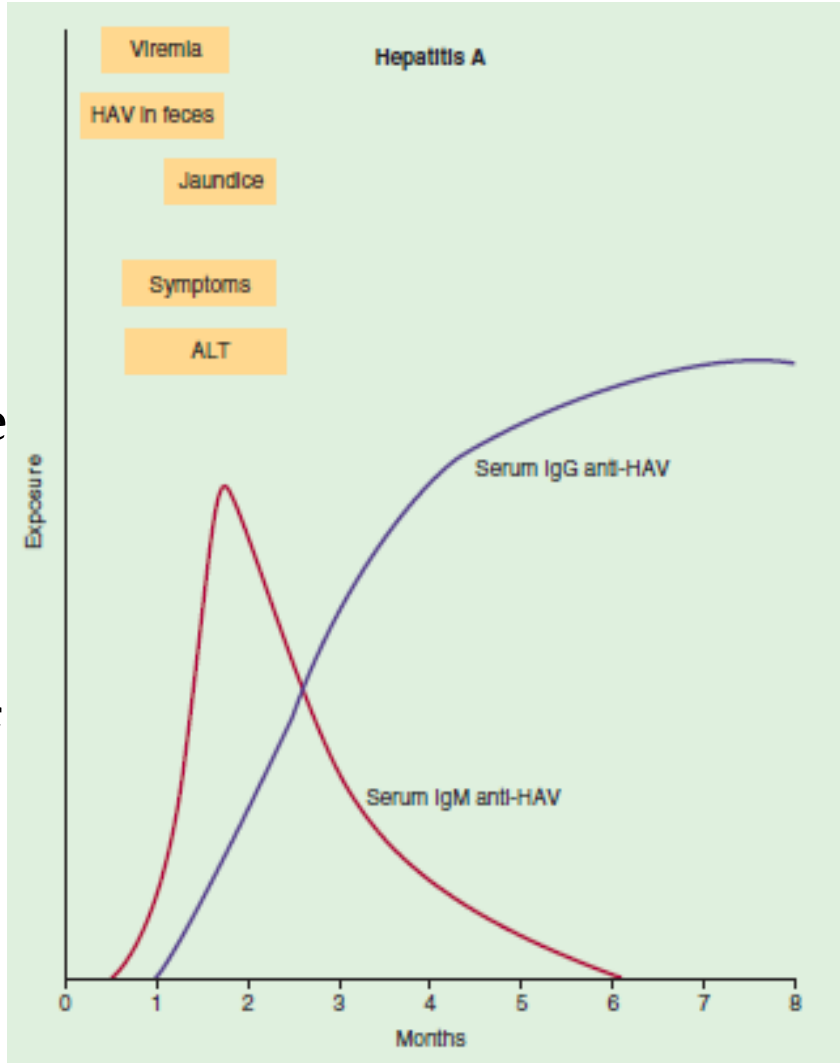
HAV
- clinical manifestations -

- Incubation period that lasts 15 to 45 days
- Followed by fever, anorexia, loss of appetite, nausea, pain in the upper right quadrant of the abdomen and jaundice
- Dark urine and pale stool
- The liver is enlarged and palpably sensitive, serum transaminases and bilirubin levels are increased
- The incidence of clinically manifest disease depends on age (20:1 in children and 1:1 in adults)



HAV
- diagnosis, treatment, prevention-

- Specific IgM antibodies are sufficient for diagnosis
- There is no specific treatment
- **Passive immunization**: hyperimmune globulins administered before and during incubation
- **Active immunization**: inactivated vaccine that induces the production of antibodies, provides protection in 100% of cases and today is recommended for all children aged one year, and in adults only for those at high risk



Hepatitis B virus (HBV)

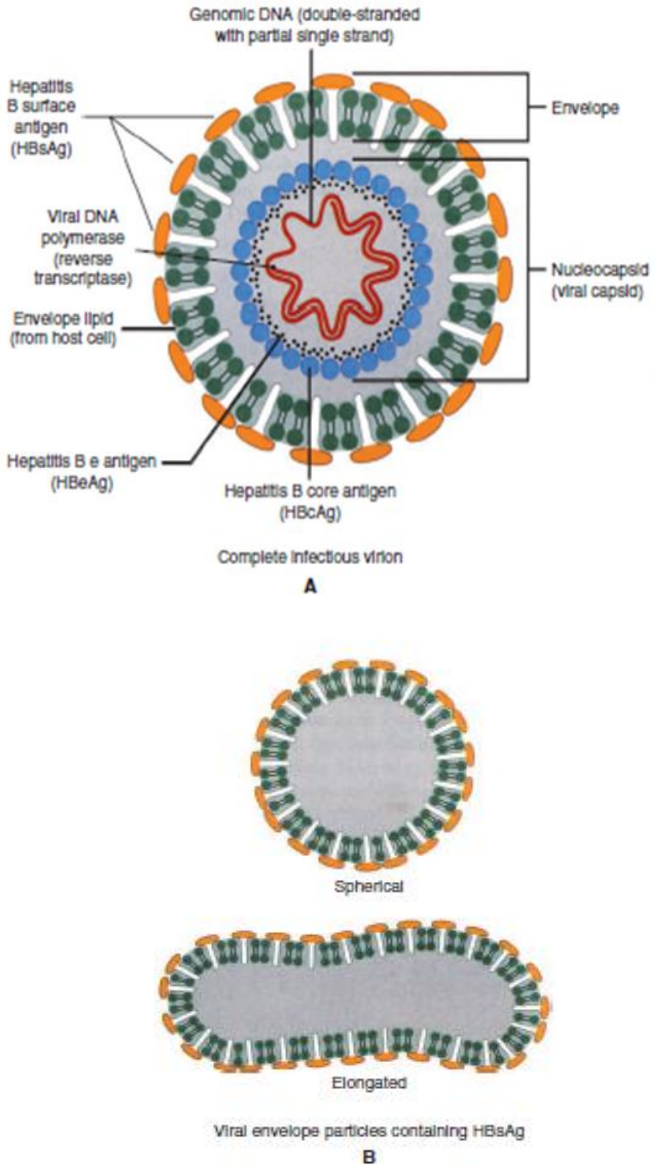
- Belongs to the family *Hepadnaviridae,* the cause of "serum" hepatitis

Complete infectious virion, ***Dane* particle,** is spherical and consists of:

Core:

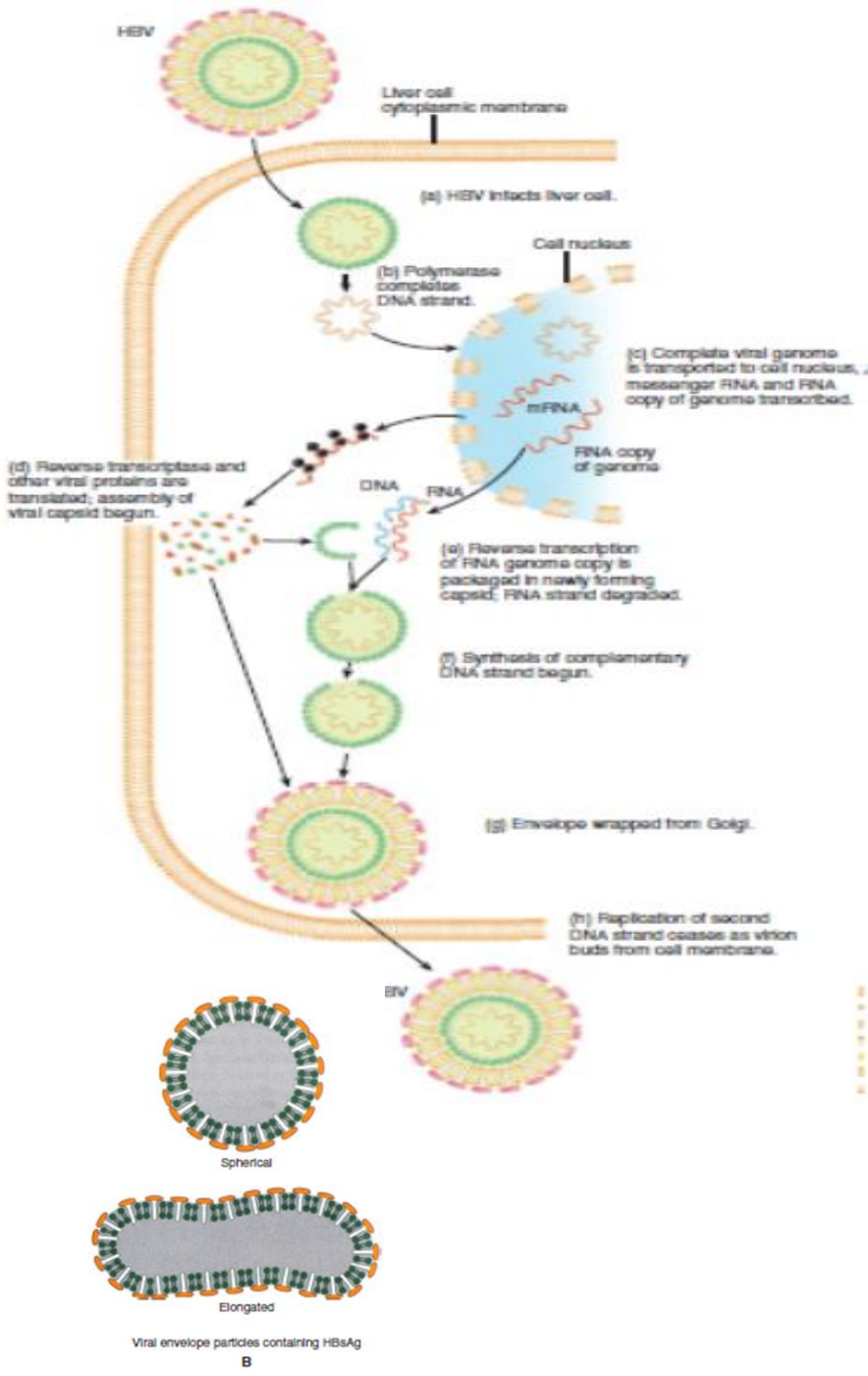
1. Nucleocapsid (partly double-stranded DNA). **viral DNA codes envelope proteins:** hepatitis B surface antigen (**HBsAg**); *core*, nucleocapsid protein (**HBcAg**); **DNA polymerase (reverse transcriptase, RNA-ase H)** and **HBx protein, a transcriptional activator**
2. **Hepatitis B antigen** or **HBeAg, a low-molecular-weight glycoprotein secreted by infected cells**

Lipid bilayer containing **HBsAg**



Hepatitis B virus (HBV) -replication-

- HBV replication involves a step of **reverse transcription** (unique among all DNA viruses)
- **HBsAg aggregates** (spherical and filamentous forms) **and HBV DNA are detected in the serum**, indicating the presence of an infectious virus
- In infected liver tissue, HBcAg, HBeAg, and HBV DNA can be found in the nuclei of infected hepatocytes, while HBsAg is found in the cytoplasm



HBV
-epidemiology-

- **Chronic infection carriers** are the major source of infection
- HBV transmission occurs through **the exchange of blood and body fluids** with the infected people
- In some countries, especially in the Far East, 5 to 15% of people carry this virus and most are asymptomatic

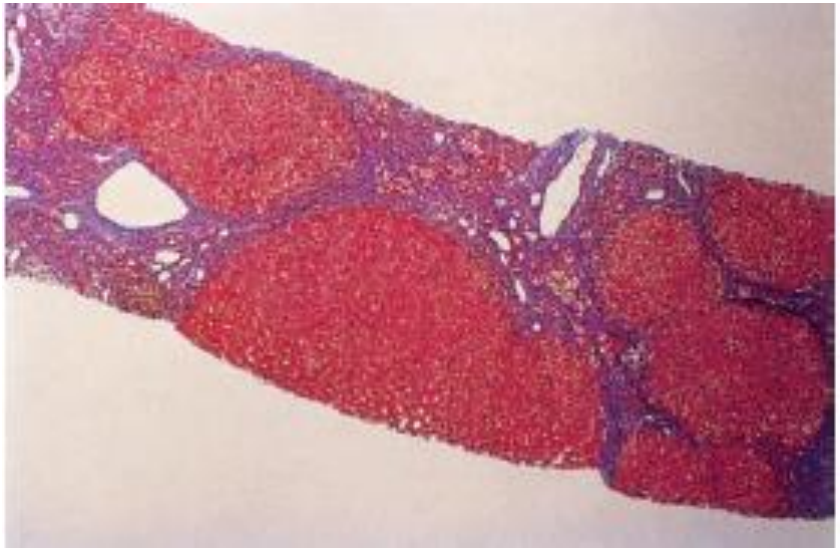
areas with high prevalence are marked in black

- **Neonatal infection** is not a consequence of transplacental transmission but **occurs intrapartum** and is usually asymptomatic - lifelong chronic carriers of the infection due to the inability to produce anti-HBsAg antibodies
- **Persistent HBV infections** are associated with the development of **hepatocellular carcinoma**

HBV
-pathogenesis-

- **Tissue damage is probably a consequence of immune reactions**
- **Cellular immunity (cytotoxic lymphocytes) is crucial for virus control, but also contributes to liver damage**
- Antibodies to HBcAg, which are present in chronic carriers of the disease that show persistent production of hepatitis B virion, are not important in protection against the virus
- Antibodies to HBsAg are protective and are associated with disease resolution !!!

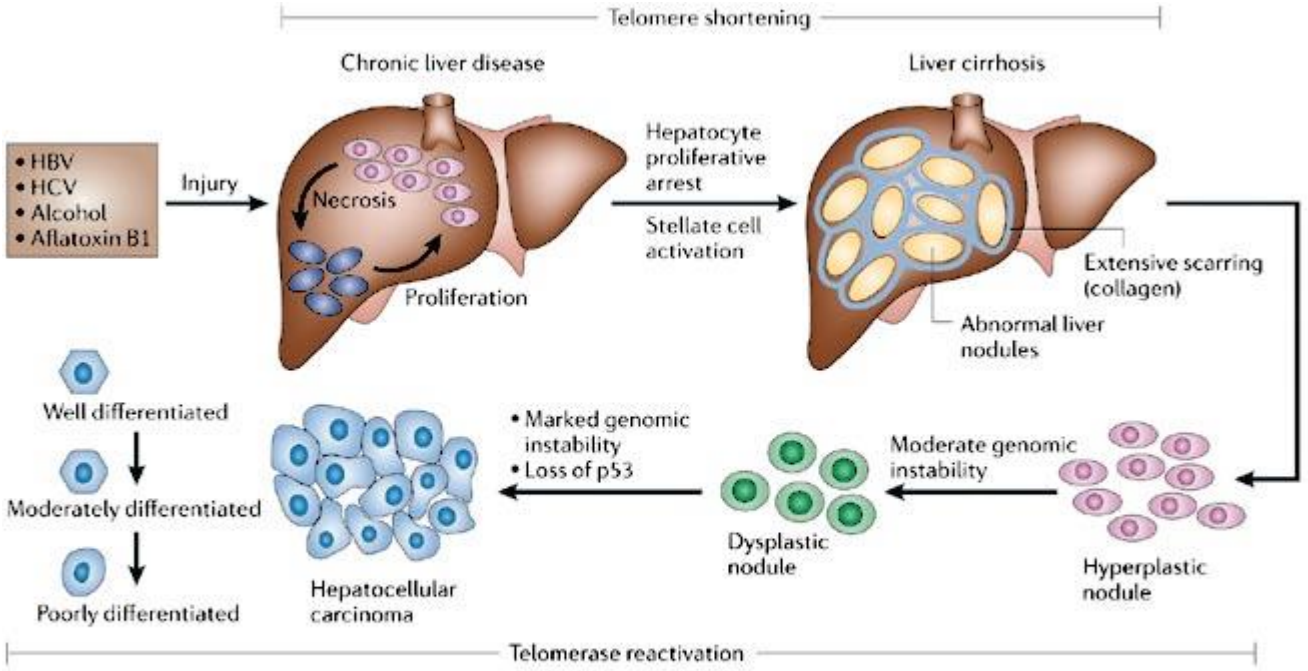
- Inflammation – Necrosis – Fibrosis



HBV
- pathogenesis -

Integrated HBV DNA can be found **in the cells of almost all hepatocellular carcinomas**

- HBV transcriptional transactivator protein HBx activates Src kinases that may affect carcinogenesis
- HBx protein interacts with the tumor suppressor gene p53, which may play a role in the development of this cancer



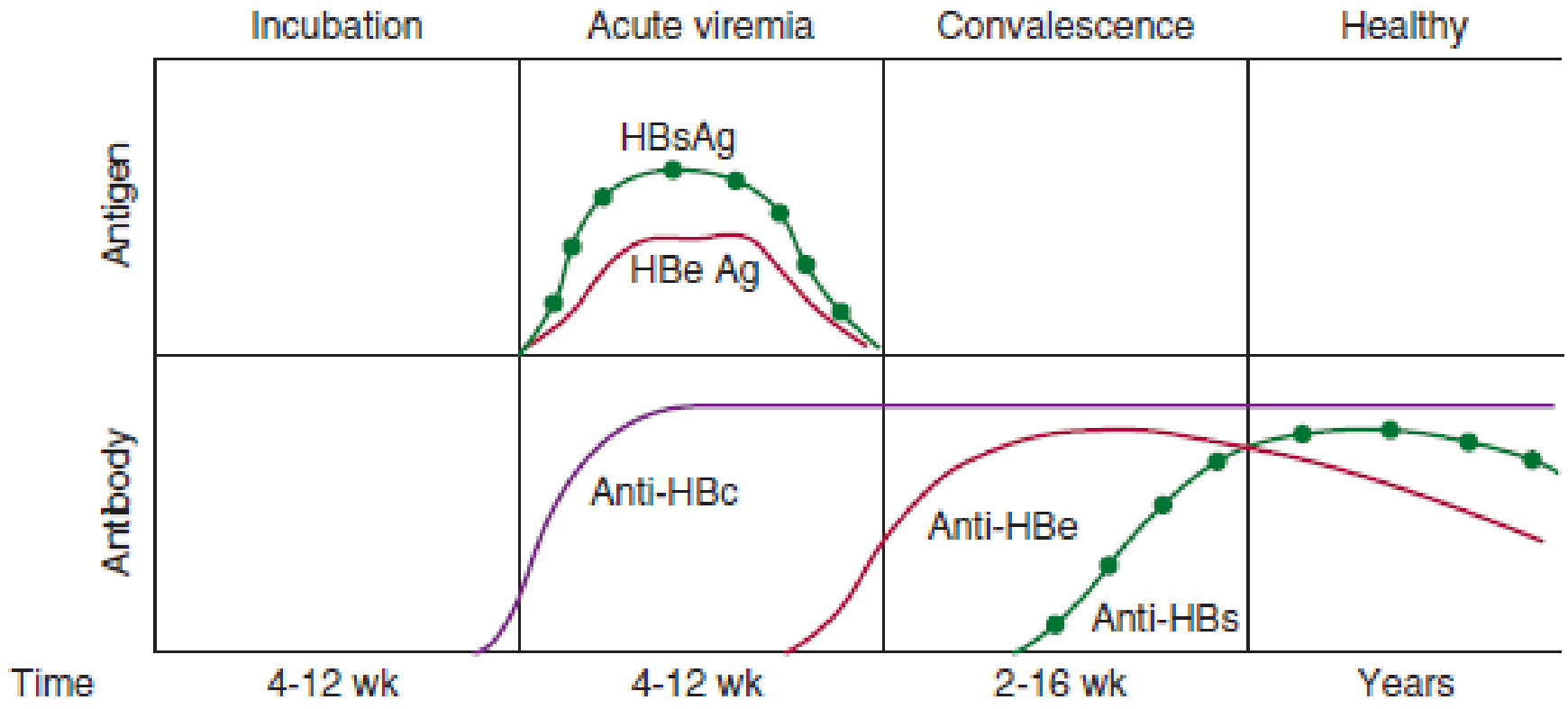
HBV
-clinical manifestations-

- Incubation period may be short, up to 30 days, but may last up to 180 days

- Possible clinical forms:
  - **Subclinical**
  - **Acute** (fatigue, loss of appetite, nausea, pain and a feeling of fullness in the upper right quadrant of the abdomen, swelling and pain in the joints, skin rash. With further enlargement of the liver, cholestasis develops and poor stool discoloration, darker urine and jaundice occur)
  - **Fulminant** (extensive hepatocyte necrosis), 1% of cases
  - **Chronic** (constant replication of the virus in the liver and the presence of serum HBsAg), 10% of cases

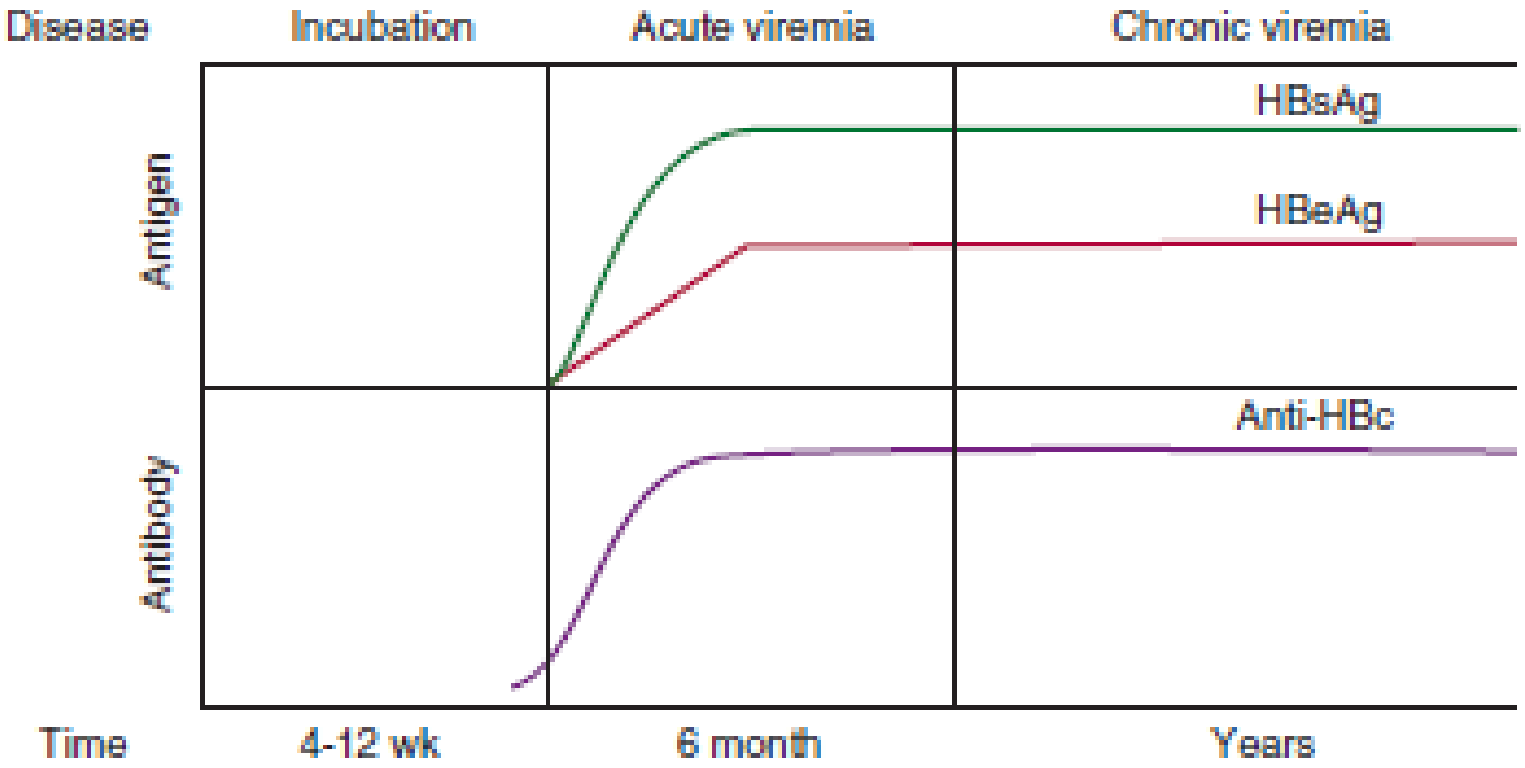
HBV
-diagnosis-

- **Acute infection**: large amounts of HBsAg and HBV DNA in the serum, and **anti-HBc antibodies IgM** (these antibodies disappear 6-12 months from the onset of acute disease)
- The appearance of anti-HBsAg antibodies indicates the elimination of the infection



HBV
-diagnosis-

- **Chronic infection**: detection of serum HBsAg in the absence of anti-HBsAg antibodies



The time of onset of serum antibodies and antigens in chronic hepatitis

HBV
-treatment and prevention-

Treatment:

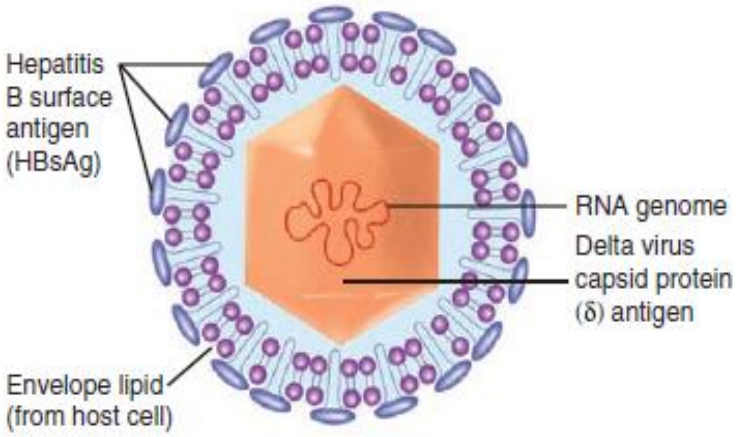
- PEG-IFN-α, lamivudine (HIV reverse transcriptase inhibitor) and nucleoside analogs (entecavir, telbivudine, adefovir) inhibit viral replication and can reduce viral load, but do not play a role in curing HBV infection

Prevention:

- Screening of donor blood and plasma products for the presence of HBsAg and anti-HBcAg significantly reduced the incidence of hepatitis B
- Hiperimmune hepatitis B immunoglobulins, HBIG for passive prophylaxis
- Purified inactivated HBsAg subunit vaccine for active immunization
- The combination of active and passive immunization is a more effective method for the prevention of neonatal infection and chronic carrier state, which is frequent in this case
- Routine testing of pregnant women for the presence of HBsAg is recommended, and in case of a positive finding, newborns should receive HBIG immediately after birth and three doses of vaccine

Hepatitis D virus (HDV)

- A small virus containing a single-stranded circular negative RNA,
- **It requires the presence of HBsAg to be transmitted from person to person and is only found in people with acute or chronic HBV infection**



- Proteins associated with circular RNA form HDV capsid antigen surrounded by HBsAg
- Because HDV does not have its own RNA polymerase, it uses cellular RNA polymerase to synthesize mRNA and genomic RNA.
- Delta capsid antigens are synthesized, associated with HDV circular RNA as it obtains the envelope of the endoplasmic reticulum and Golgi apparatus containing HBsAg

Hepatitis D virus (HDV)

Countries where 10% of people infected with HBV also have HDV infection

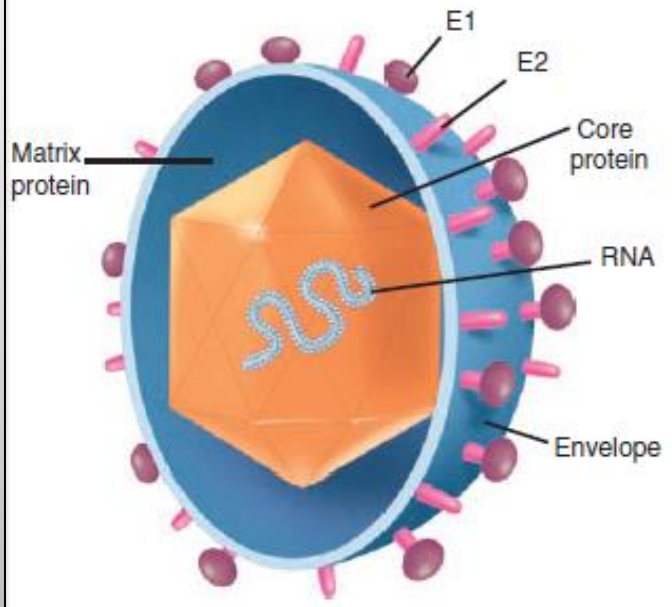
- **Simultaneous infection with HDV and HBV** results in the development of clinical hepatitis that is not different from acute hepatitis A and E, but may manifest as a secondary increase in AST, ALT

- **HDV superinfection** in people with chronic hepatitis B is characterized by relapse of jaundice and a much higher chance of developing chronic cirrhosis

- Diagnosis of infection is made by detecting IgM or IgG antibodies, or both, to serum delta capsid antigen
- Treatment and prevention are the same as for HBV hepatitis

Hepatitis C virus (HCV)

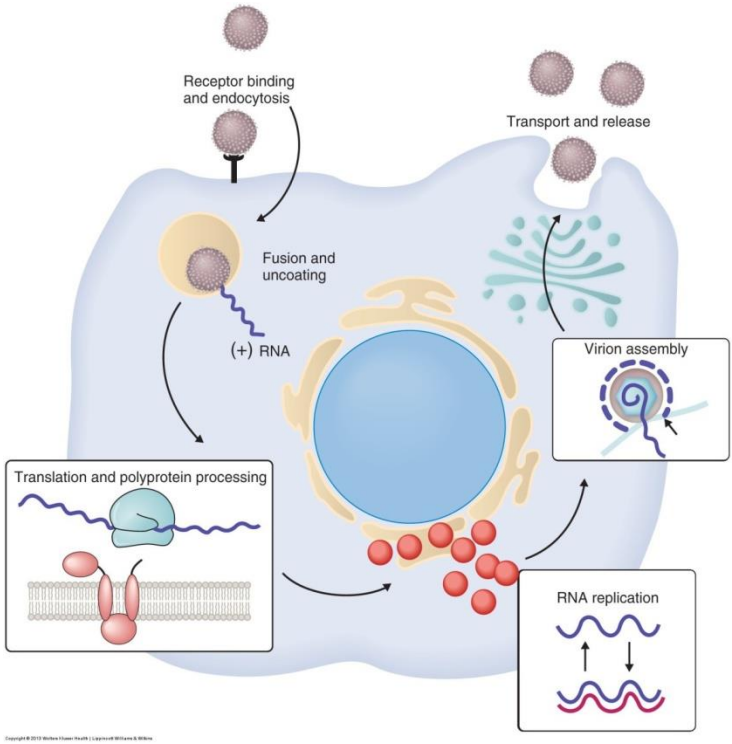
- RNA virus, in the family *Flaviviridae*, genus *Hepacivirus*
- Icosahedral capsid or core (C) protein
- Lipid bilayer envelope with two typical viral glycoproteins E1 (gp31) and E2 (gp70) that interact with receptors and coreceptors on host cells (Antibodies to these glycoproteins are involved in virus neutralization)



- **The HCV genome is very prone to mutations** (E2 glycoprotein) that allow the virus to evade the host immune system and cause chronic persistent infections
- There are at least 11 genotypes and many subtypes of HCV

HCV
-replication-

- In the human body, HCV forms a complex with lipoproteins – lipoviroparticle (LVP)
- LVPs bind to hepatocyte proteoglycan heparan sulfate and further to low molecular weight lipoprotein (LDLR) receptors resulting in unproductive infection
- In productive infection, the E2 glycoprotein interacts with the scavenger receptor B and the CD81 molecule; the virus enters hepatocytes by receptor-mediated endocytosis
- Like other positive RNA viruses, HCV replicates in the cytoplasm of infected cells
- RNA-dependent RNA polymerases, structural (C, E1 and E2) and non-structural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) viral proteins are synthesized in the form of polyproteins which are then cleaved to mature proteins by viral and cellular proteases in the cytoplasm



HCV
-epidemiology and the mode of transmission-

- Worldwide, about 150 million people have chronic HCV infection, and 3 to 4 million are infected annually
- The highest prevalence is in the Middle East, especially in Egypt

Mode of transmission:

- HCV is, as HBV, spread **parenterally, through blood** (prior to the introduction of blood screening, transfusion was the main route of transmission for hepatitis C.)
- **Sexually** transmitted disease (less frequently than HBV)
- Transmission **by contaminated needles** (40% of cases)
- **Vertical transmission** during childbirth

HCV
-pathogenesis-

- HCV infects B and T lymphocytes and peripheral blood monocytes and is transferred to the liver
- The replication rate in hepatocytes is very high
- **Liver damage** is partially a consequence of **direct cytopathic effect of the virus** and mainly **the immune response to the virus**
- **Immune complex formation**: **arthritis**, **vasculitis** and **glomerulonephritis**

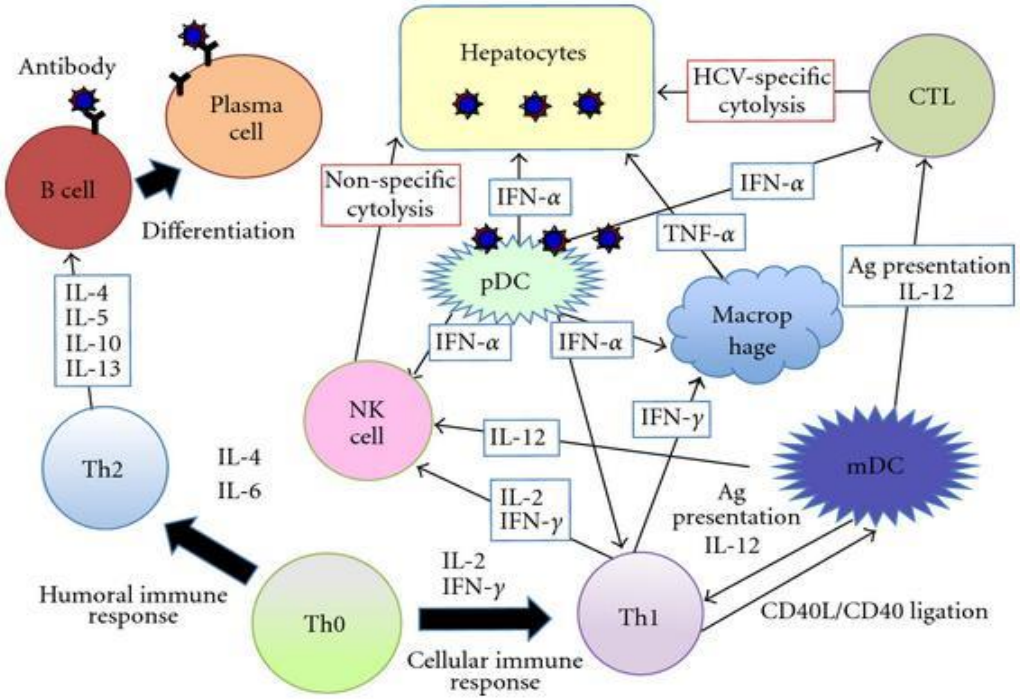
Impaired cellular immune response significantly increases the risk of developing a chronic form of the disease

Chronic HCV disease bears a risk for developing **liver cirrhosis** and **hepatocellular carcinoma**

HCV
-immune response-

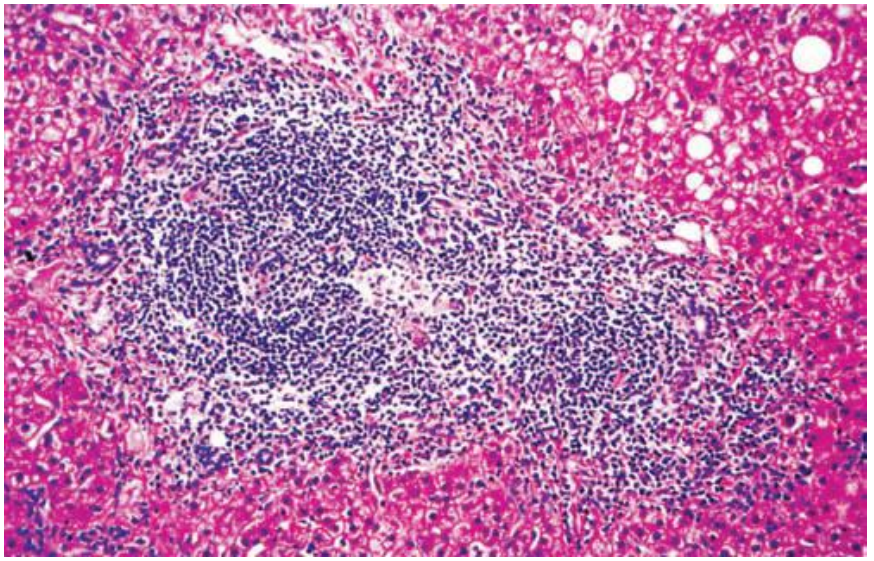
- **Mutations** of E1 and E2 proteins enable the virus to **evade humoral immune response** and establish a persistent infection
- **Anti-HCV antibodies – immune complex formation**
- **Any deficiency of cytotoxic lymphocyte responses is associated with the occurrence of chronic infection**
- Chronic infection is probably due to an imbalance between Th1 and Th2 cytokines

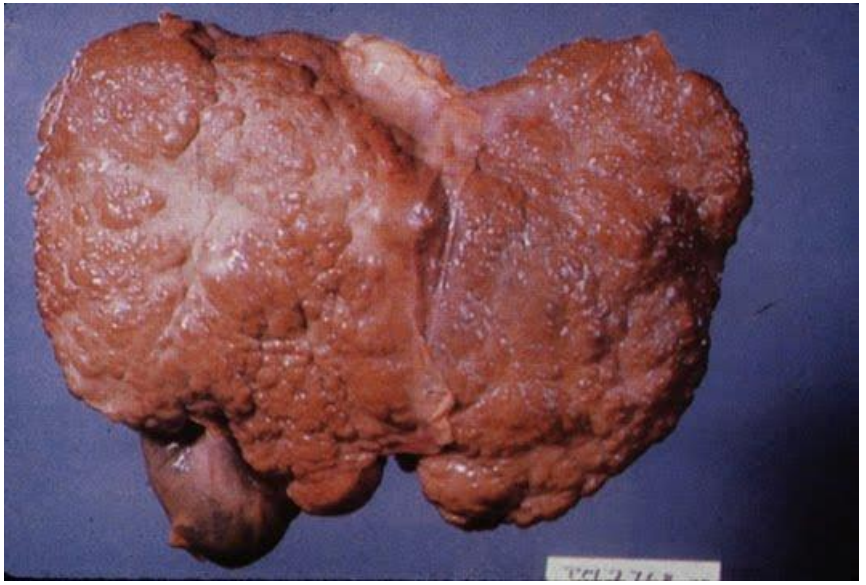
- **Pronounced production of TNF-α** causes hepatocyte damage and chronicity
- **Genetic factors** contribute to the development of chronic infections: the DR5 allele is associated with a lower incidence of cirrhosis, and the HLA A2 restricted response of cytotoxic lymphocytes is shown in 97% of people with chronic hepatitis C.



HCV
-clinical manifestations-

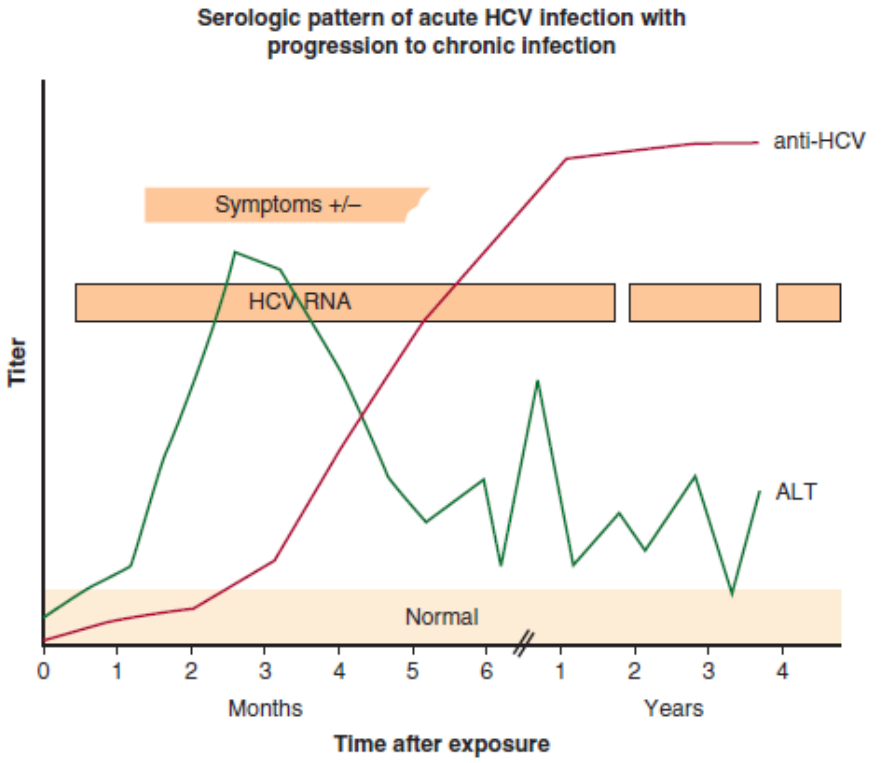
- Incubation period for HCV is 6 to 12 weeks
- HCV **generally does not cause clinically evident acute disease**, **mild chronic hepatitis** that eventually leads to liver failure develops in about 25% of the infected
- Fulminant HCV hepatitis is quite rare
- The median time from infection to the development of chronic hepatitis is 10 to 18 years
- **Cirrhosis** and **hepatocellular carcinoma** are late sequelae of chronic hepatitis





HCV
-diagnosis, treatment and prevention-

- The humoral immune response to HCV does not develop in acute disease for the first three weeks after the onset of clinical symptoms, so RT-PCR is the method of choice for HCV detection
- Combination therapy with IFN-α and ribavirin is the treatment of choice for chronic forms
- Blood product screening is a very important prevention measure
- Prophylactic use of intravenous immunoglobulins does not provide protection against hepatitis C

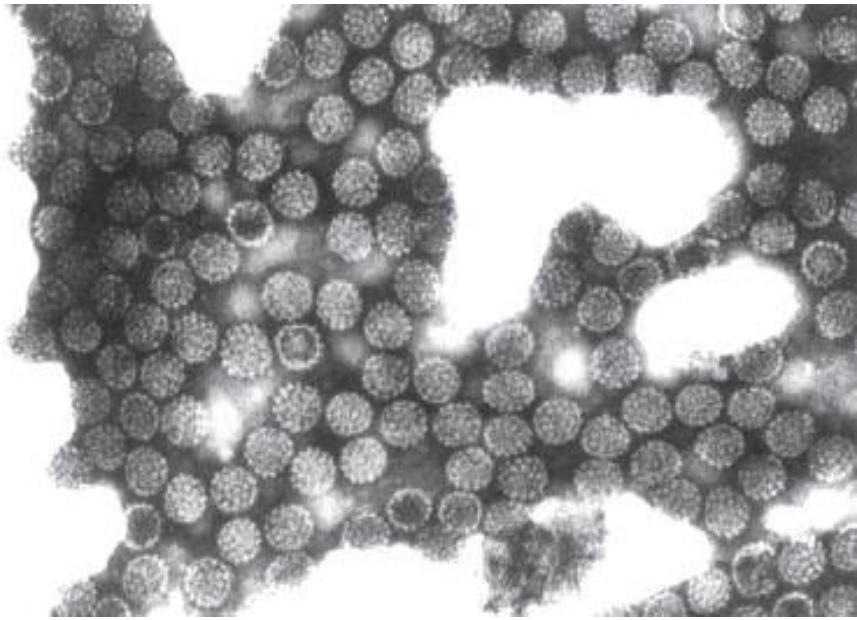


Hepatitis E virus (HEV)

- Positive, single-stranded RNA, icosahedral nucleocapsid, nonenveloped virus
- It is transmitted by **fecal-oral route**, usually by contaminated drinking water, but transmission by ingestion of infected animal products is also possible. Transmission from animals to humans, by **transfusion** of infected blood products, as well as **vertical transmission** are also possible.
- The incubation period for hepatitis E is approximately 40 days
- The infection is very often **subclinical**, and symptomatic infection is characterized by an **acute illness** that **can be fatal**, especially **in pregnant women**
- Diagnosis is made by detecting specific IgM antibodies in serum and by detecting viral RNA by RT-PCR analysis
- There is no specific treatment

Family *Papillomaviridae*
Papilloma virus

- Small nonenveloped viruses with nucleocapsid containing double-stranded DNA and icosahedral symmetry



- Double-stranded DNA of human papillomavirus codes 7 or 8 early genes (E1-E8) and two late structural capsid genes (L1 and L2)

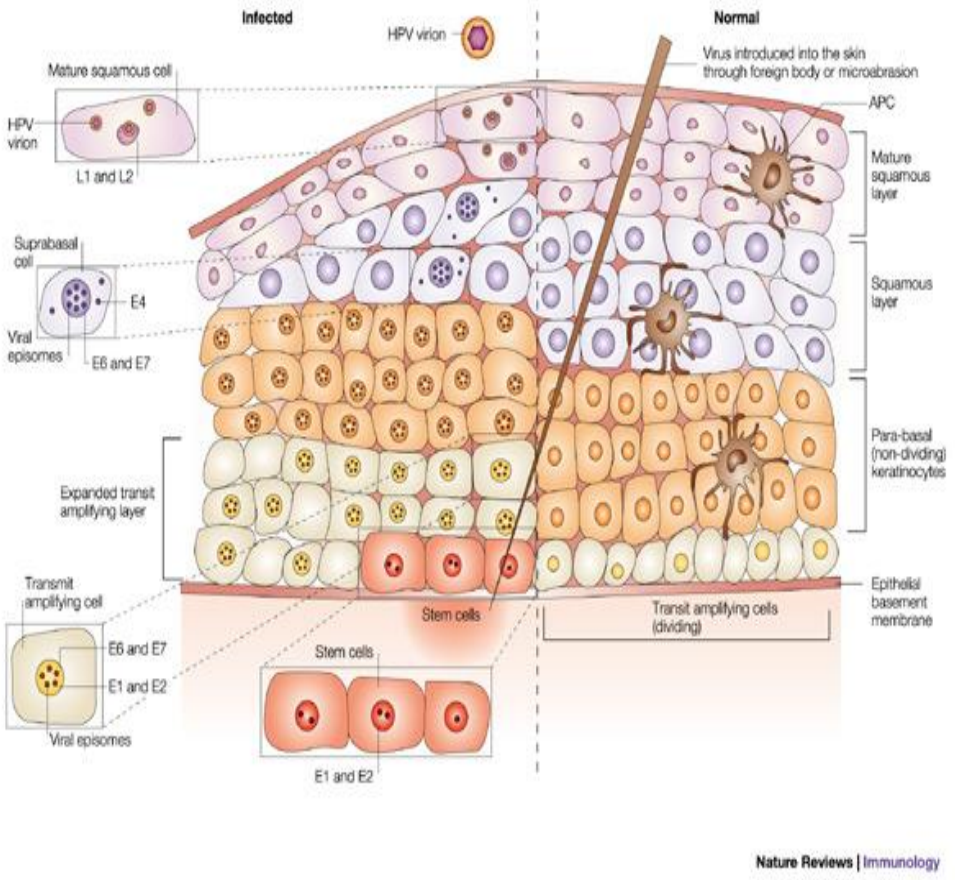
- Early genes are in charge of regulation of viral replication and transformation
- The virus does not encode polymerases, so it depends entirely on the transcription of the infected cell
- Based on DNA homology, HPVs are classified into about 100 genotypes
- Different group members are species-specific

Papilloma virus -replication-

- **HPVs establish the infection in the basal cell layer of the epithelium**, they are not lytic, as they do not kill basal cells upon entry (latent infection)

- Heparan sulfate proteins mediate binding to host cells. Upon entry, one or, at most, several copies of viral DNA are maintained as extrasomal nuclear episomes

- The viral genome initially replicates as an autonomous episome synchronously with cell chromosomes



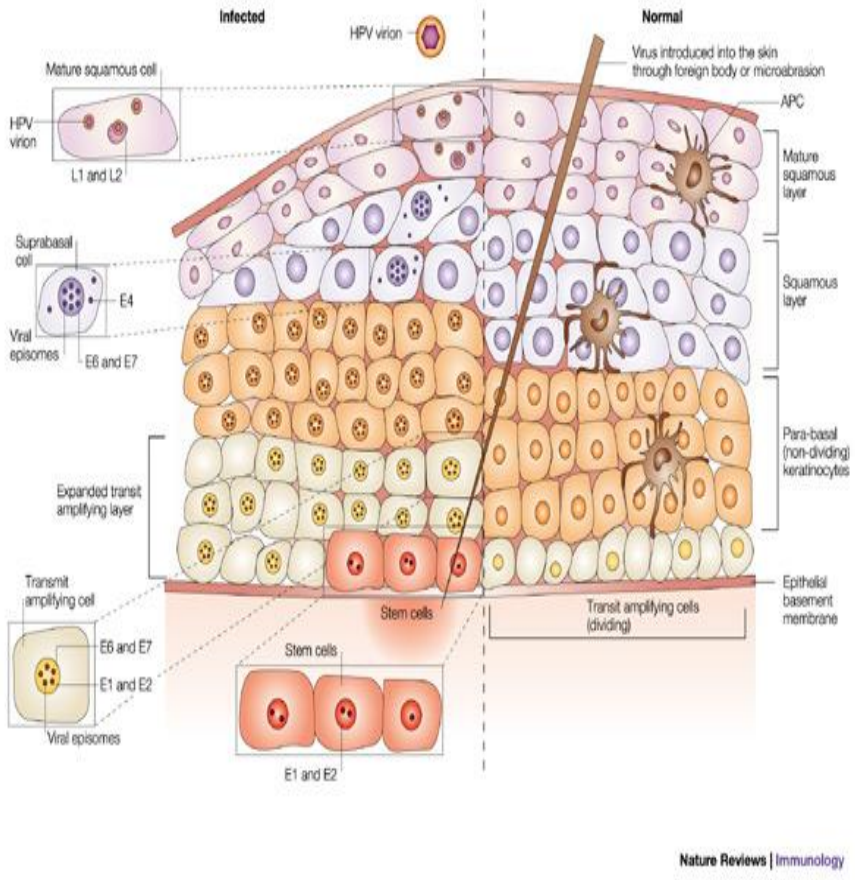
Papilloma virus
- replication-

- As cells mature and migrate to the surface of the skin, the viral genome is transported to the upper layers of the epithelium where additional viral genes are expressed and more intense amplification of the viral genome begins

- Viruses respond to signals from terminally differentiated keratinocytes and initiate transcription of late mRNA encoding L1 and L2 capsid proteins

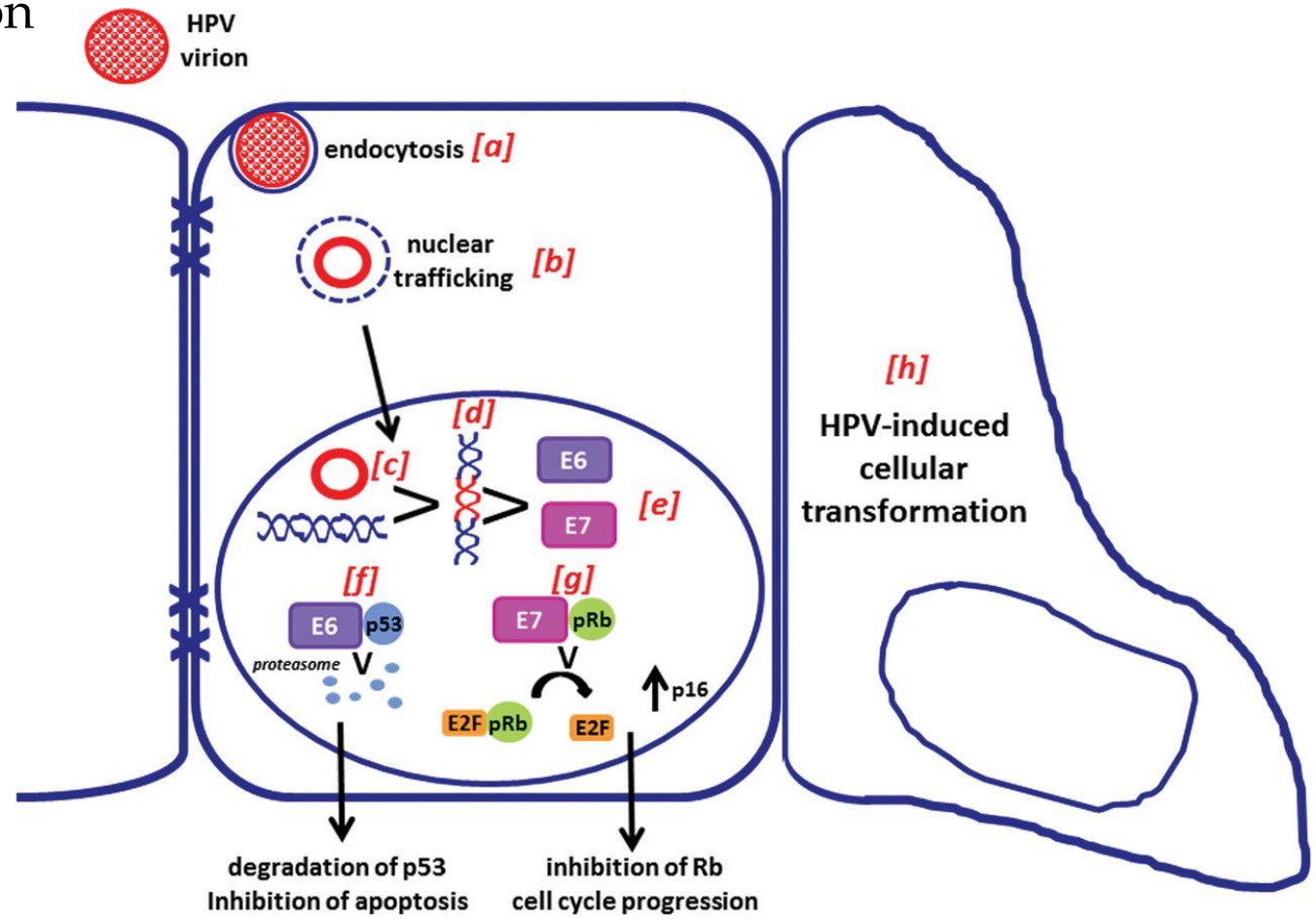
- These late proteins are transported to the nuclei of keratinocytes where they assemble to form hundreds of thousands of capsids

- Newly formed virions are released from the surface of the epithelium



Papilloma virus -oncogenic potential-

- The products of the early E6 and E7 genes are involved in cell transformation
- E6 binds to p53 and E7 to pRb protein, thereby disrupting apoptosis and cell cycle regulation

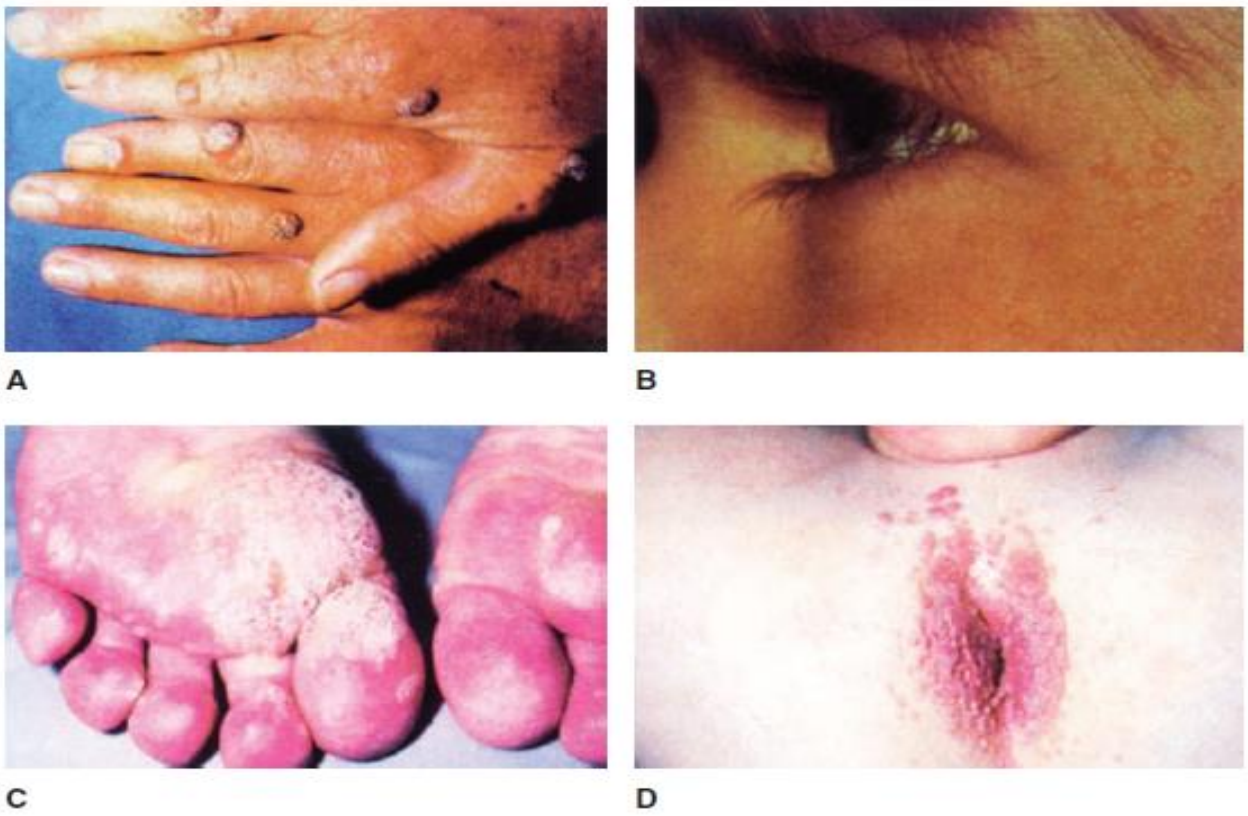


Papilloma virus

- the most common sexually transmitted infection
- spread by direct skin-to-skin contact
- Asymptomatic disease carriers
- HPV DNA can be found in more than 95% of cervical cancer samples

Lesions associated with HPV	
Lesions	**HPV types**
Plantar and palmar warts	HPV -1, 4
Common cutaneous warts	HPV -2, 4
Flat warts	HPV -3, 10
Anogenital warts (condyloma), respiratory papillomatosis, cervical papillomas; “low risk" for becoming malignant	HPV -6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81
Epidermodisplasya veruciformis, immunosuppression	HPV -5, 8, 9, 12, 14, 15, 17, 19-29
Penile, vulvar, vaginal, and anal warts, Bowenoid papulosis, high risk for cervical and anal dysplasia and carcinoma	HPV -16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73

HPV
-clinical manifestations-



The cell-mediated immune response is thought to be essential for limiting HPV

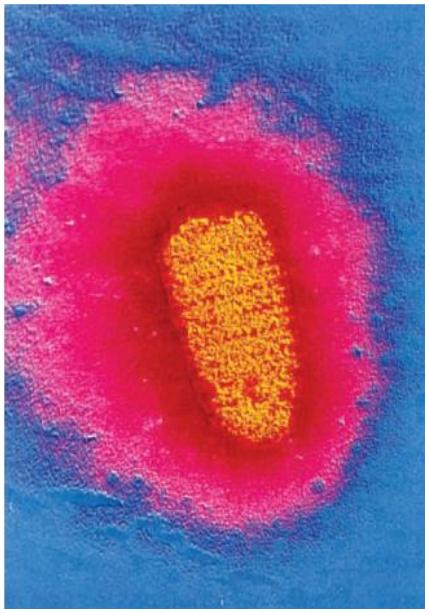
- People with cellular immunity deficiency are more susceptible to developing chronic warts that are resistant to therapy
- Some people have an impaired response to only one or a few types of HPV and may have extensive warts that often cover both hands and feet but are not susceptible to other viral or bacterial infections

HPV
-prevention-

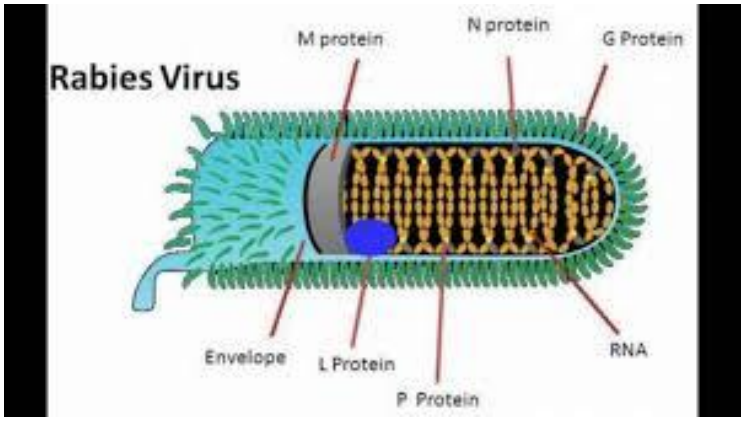
- Regular examinations of the external genitalia, vagina, and cervix including Pap tests
- Cesarean section if the mother has an active infection
- Prophylactic vaccines to prevent HPV infection (contain only viral proteins of HPV 16 and 18)

Rabies virus

Rabies (*Lyssa*) is an acute fatal disease of the central nervous system



- Rabies virus belongs to *Rabdoviridae* family, genus *Lyssavirus*
- It has a bullet shaped, helix nucleocapsid with a single-stranded negative RNA and RNA-dependent-RNA polymerase packaged in a matrix protein coated with a glycoprotein-containing bilayer
- G protein of the virus binds to acetylcholine or to neural cell adhesion molecule (NCAM receptor) present on the cell surface



Rabies virus -epidemiology-

Rabies exists in two epizootic forms:

- **urban** - associated with the outbreak of the virus in non-immunized domestic animals, dogs and cats
- **silvan** – occurs in wild foxes, wolves, raccoons and bats, but is not found in rodents or rabbits

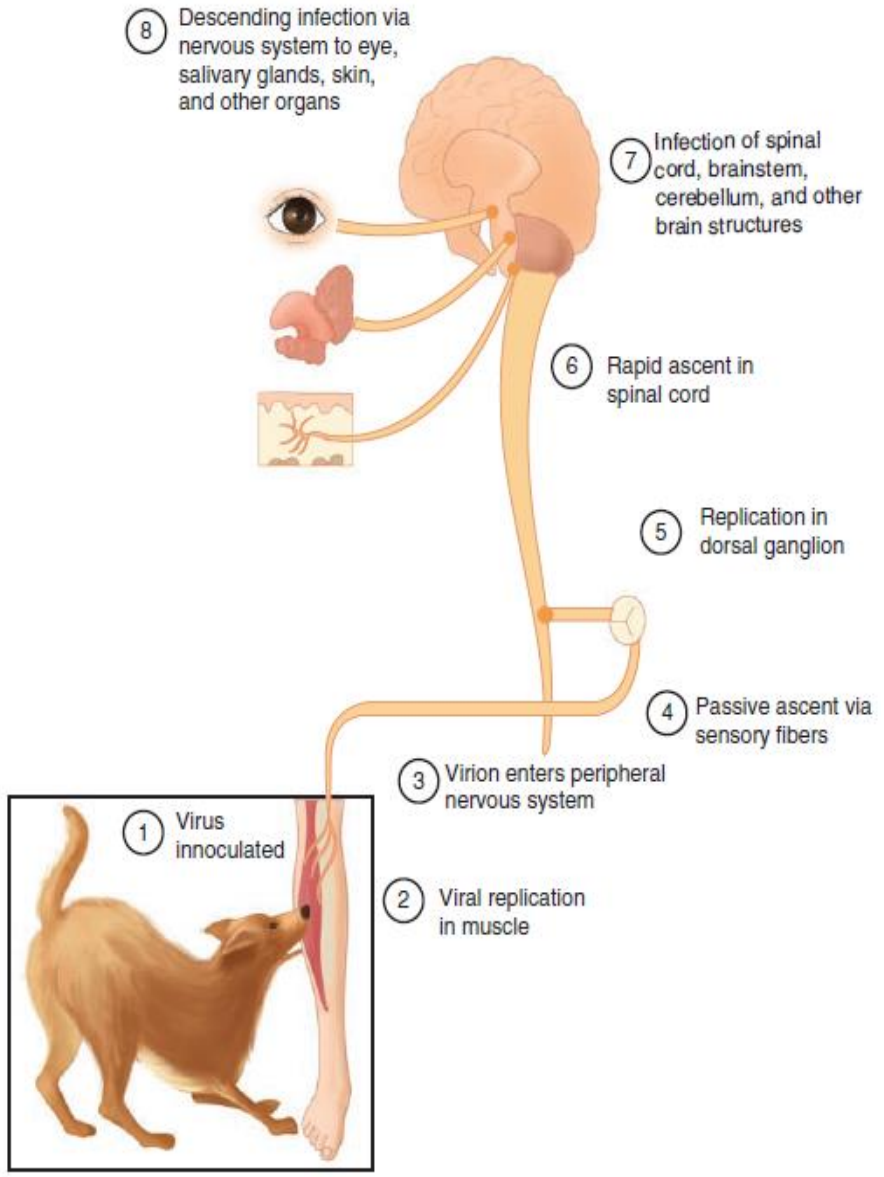
In developing countries, rabies is most commonly transmitted by the bite of domestic non-immunized animals or mice, and in the developed countries by the bite of wild animals.

In previous years, cases of virus transmission by aerosol in bat caves have been described

Human-to-human transmission has been demonstrated only in cases of corneal and organ transplant. Infected people could potentially transmit rabies by bite, but no such cases have been reported

Rabies
-pathogenesis-

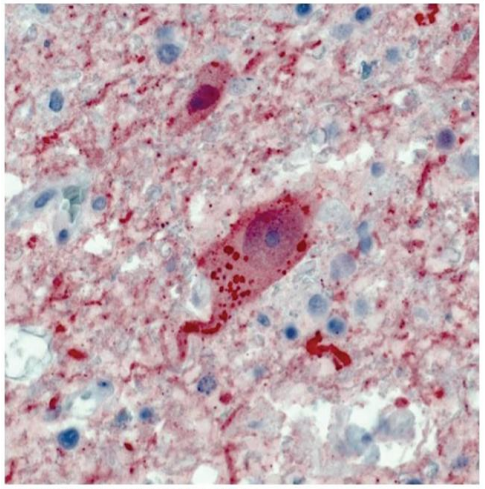
- The incubation period lasts between 10 days and one year, and depends on the amount of virus inoculated, the amount of tissue affected, the host's immune response, innervation at the site of the bite, and the distance the virus must pass from the site of entry to inoculate the CNS
- The virus **first replicates in muscle tissue at the site of inoculation** and if a person does not have specific antibodies, the virus **enters the peripheral nervous system in the area of the neuromuscular junction and spreads within the CNS where it replicates exclusively in gray matter**
- This is followed by centrifugal spread along the autonomic nerves to other tissues including the salivary glands, adrenal medulla, lungs and kidneys
- Spread to the salivary glands in animals facilitates the further transmission of this disease through infected saliva

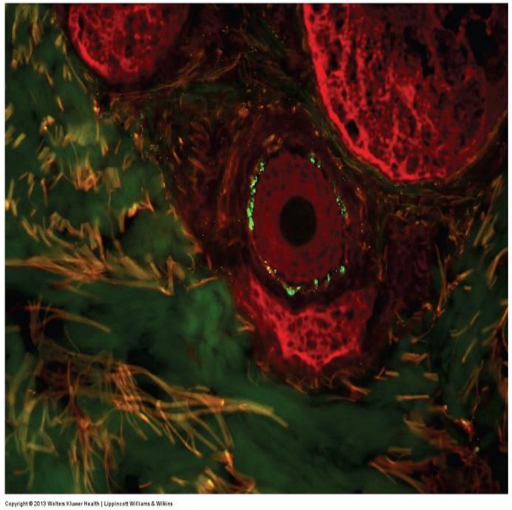


Rabies
-pathogenesis-

- The neuropathology of rabies is similar to other viral diseases of the CNS and is characterized by infiltration of CNS tissues by lymphocytes and plasma cells and destruction of nerve cells
- Immunization in the early incubation period prevents the development of infection

Pathognomonic lesions called **Negri bodies** (eosinophilic cytoplasmic inclusions mainly in the hippocampus, cerebral cortex, cerebellum and basal spinal ganglia)





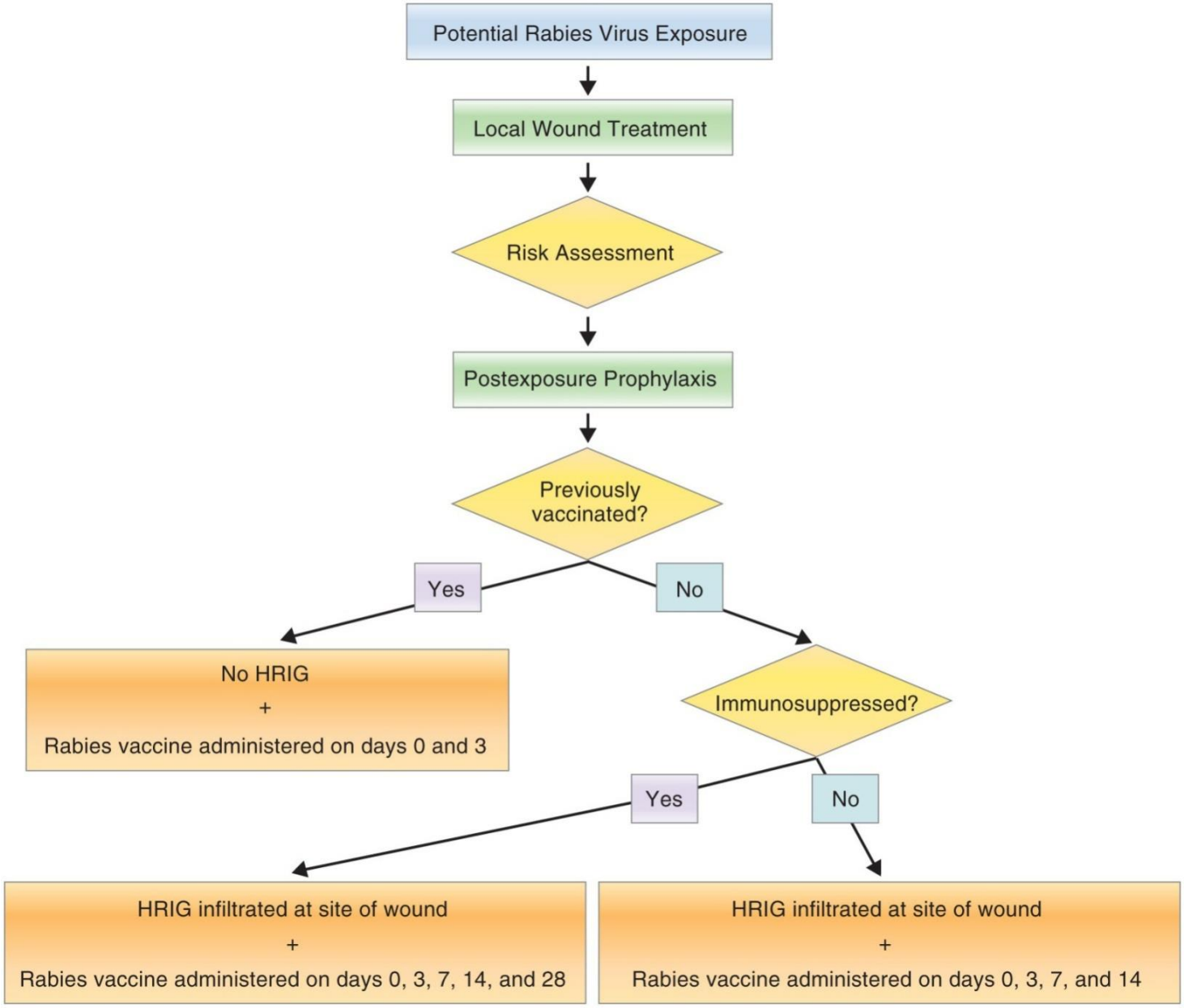
Rabies
-clinical manifestations-

Clinical stages of rabies			
Phase	**Time frame**	**Symptoms**	**Virus replication site**
Incubation	10-365 days (average 20-90)	No symptoms	Bite site, muscle cells
Prodromal stage	2-10 days	Nonspecific: nausea, headache, fever, vomiting, mild mental disorders (insomnia), pain, itching, tingling at the site of the bite	Viral replication in the CNS
Acute neurological stage	2-7 days	Furious stage: hyperreactivity, irritability, disorientation, hallucinations, bizarre behavior, hydrophobia, convulsions Paralytic stage: lethargy, paralysis (respiratory)	Replication in the brain and spread to peripheral organs
Coma	0-14 days	Coma, respiratory muscle paralysis, cardiac arrest, drop in blood pressure, secondary infections	
Death		Survival is extremely rare	

Rabies
-diagnosis-

- Key questions:
  1. Did the bite result in a skin break?
  2. Has rabies been reported in the state or region where the bite occurred?
  3. Was the biting animal rabid? Is it available for laboratory diagnosis, or did it escape?
  4. Is the species known commonly to be infected with the virus?
  5. Is the biting animal a dog, cat or ferret that can be observed? (If so, the period of shedding or potential virus transmission is usually concomitant with illness, or a few days before, up to maximum of 10 days, which allows a generous safety margin. If a person has been bitten by a dog 11 days before the dog gets sick, no prophylaxis is needed).

Rabies prophylaxis



Prions
Subacute spongiform encephalopathies

Prions

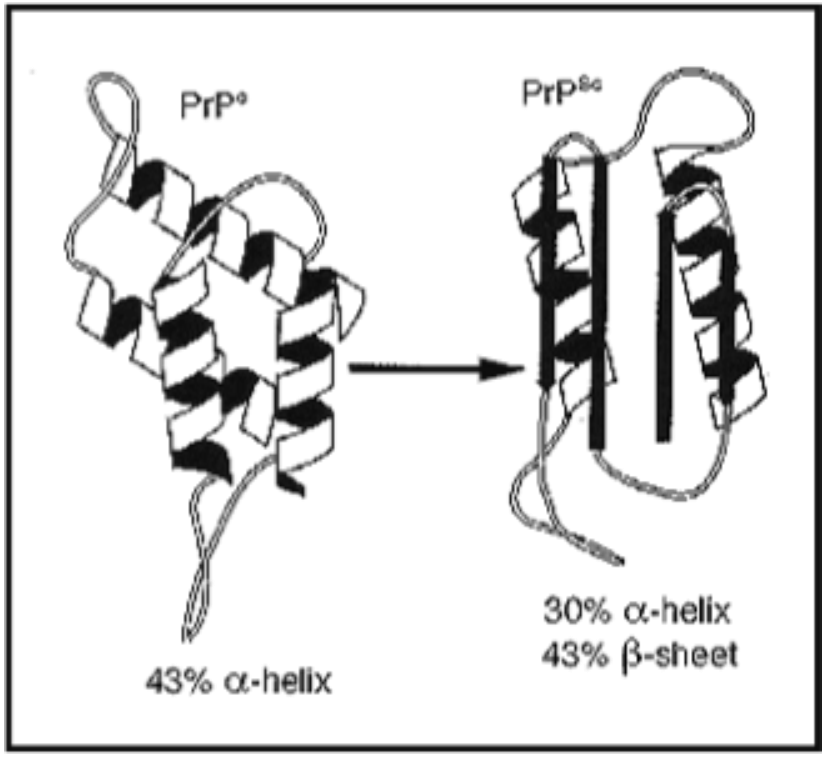
- *Stanley Prusiner* was awarded the Nobel Prize in 1997 for his work on the identification and the role of prions in disease

Prion is a small infectious particle of protein structure, 5-100 nm in diameter or less

- It cannot be inactivated by procedures that destroy nucleic acids
- It is resistant to ionizing radiation, cooking and most disinfectants
- It can remain viable in brains that have been in formalin for many years
- It cannot grow in cell culture

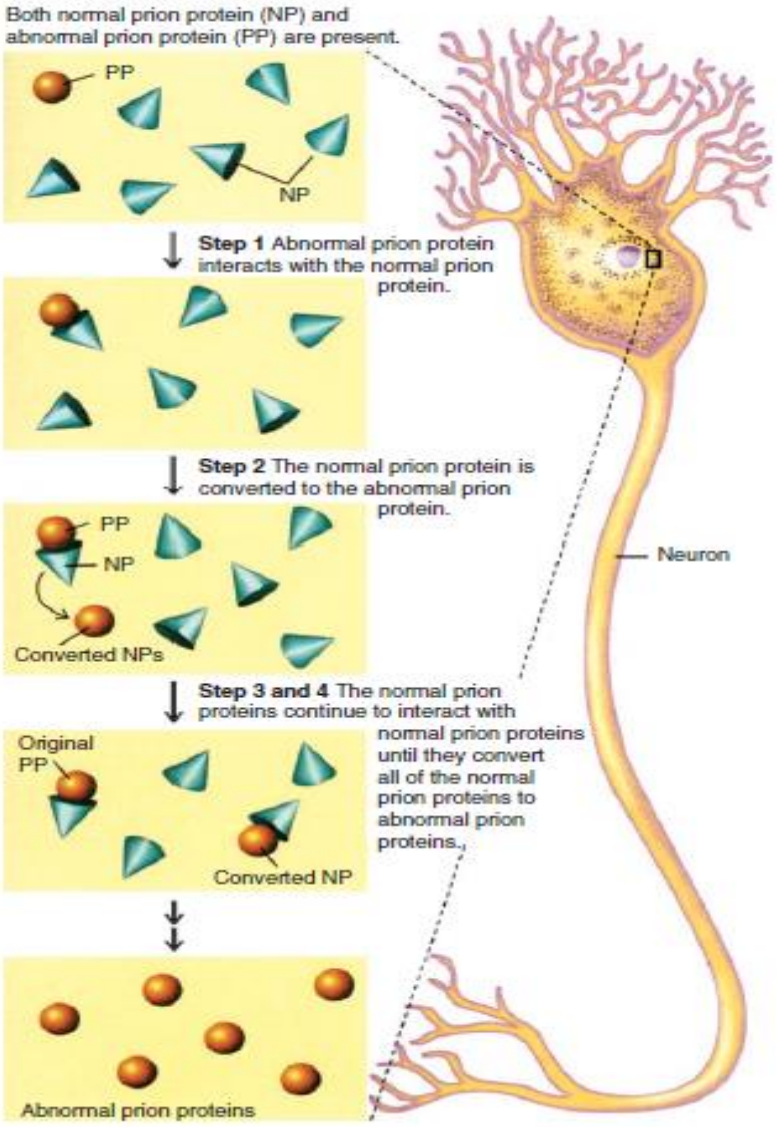
Prions

- Prion consists only of a protein that encodes a normal cellular gene, PrP, in the brain (the gene is located on chromosome 20). The protein labeled PrPc is **a protein converted to a disease-causing form** by **post-translational modifications that alter the conformation.**
- The altered protein is called PrPsc



Prions

- Changing the conformation of proteins also affects the formation of aggregates that form amyloid-like structures
- Brain extracts from animals suffering from these diseases contain an abnormal form of protein that is not found in healhy animals
- PrPsc is responsible for the transmission of infection



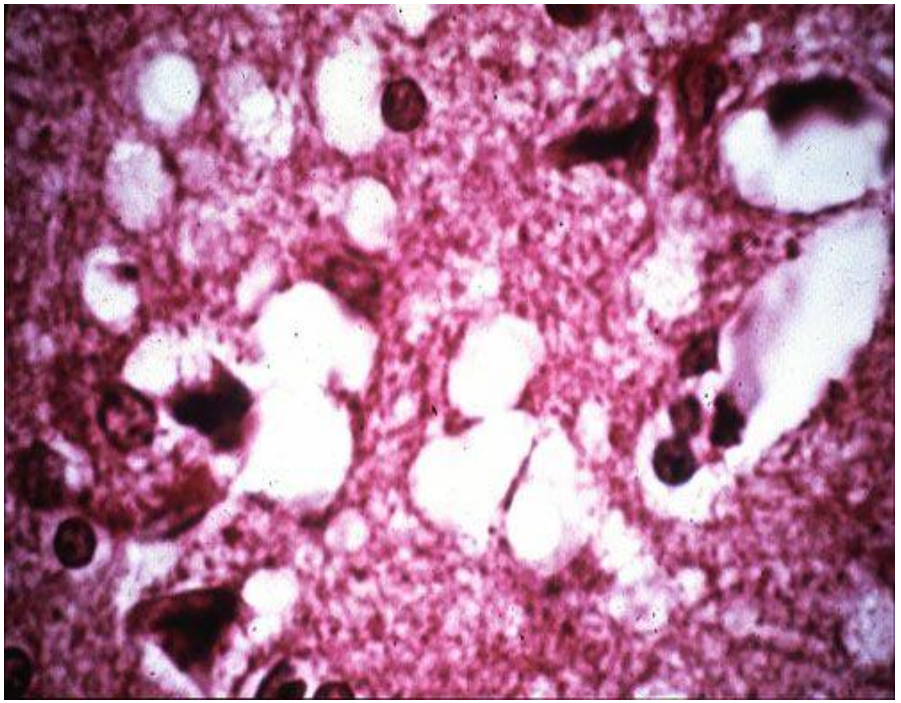
Prion diseases

- Prions are etiological agents that can be **inherited**, **infectious** or occur **sporadically**
- The pathogenesis of this disease is not well understood
- Pathological and clinical characteristics are similar (variable neuronal damage and astrocyte proliferation)
- The disease is marked as **transmissible spongiform encephalopathy** because of **vacuolar changes that occur in the cortex and cerebellum**
- Incubation lasts for months or years, and the disease ends fatally

Prion diseases	
People	**Animals**
Creutzfeldt-Jakob disease	Foot-and-mouth disease (sheep)
Variant *Creutzfeldt-Jakob* disease	Transmissible mink encephalopathy
Kuru	Bovine spongiform encephalopathy
Fatal familial insomnia	

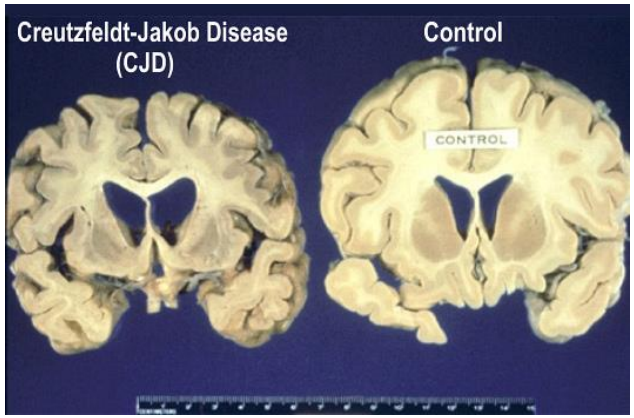
Kuru

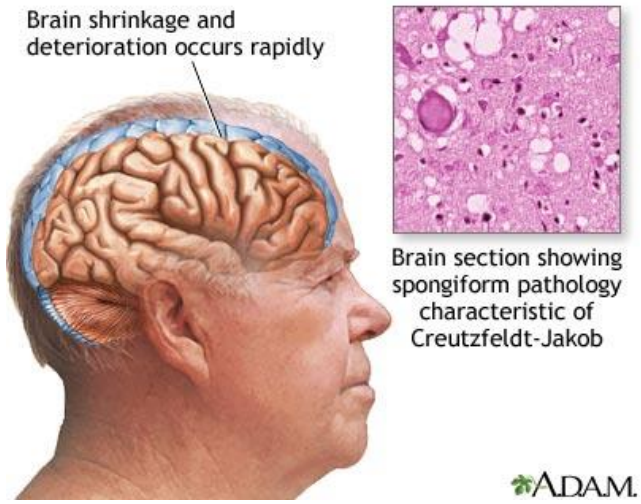
- Progressive neurological disease reported in New Guinea.
- Symptoms and signs include ataxia, hyperreflexia, spasticity, progressive dementia, and death
- Changes occur only in the CNS in the form of diffuse degeneration of neurons and spongiform changes in the cerebral cortex and basal ganglia, without the inflammatory response
- Inoculation of infected brain tissue into primates caused a disease that had shown similar neurological symptoms and pathological changes after an incubation period of approximately 40 months
- Clinically, the disease occurred 4 to 20 years after exposure
- Since cannibalism is eradicated in New Guinea, Kuru disappears



Creutzfeldt-Jakob disease

- It occurs worldwide with an incidence of 1 per million people annually
- **The mode of transmission is unknown**, it occurs **sporadically** in 85% of cases, and in 15% of cases it is **hereditary**
- Infection can be transmitted by dura mater and cornea **transplants**, **contact with contaminated electrodes and instruments** used in neurosurgical procedures, as well as **growth hormones** derived from the pituitary glands
- It usually occurs in 6th and 7th decade of life
- Initial clinical manifestations are changes in cerebral function, forgetfulness and disorientation progress to severe dementia worsening over 4-7 months, followed by paralysis, pneumonia and death





Creutzfeldt-Jakob disease
-prevention-

- There is a small risk of hospital-acquired infections
- Stereotactic neurosurgical instruments, especially those used in people with undiagnosed dementia, should not be re-used.
- Organs from people with undiagnosed neurological diseases should not be used as transplants
- Growth hormone from human tissue has been replaced by recombinant proteins
- Recommendations for disinfection of potentially infectious material

Mad cow disease and a variant of *Creutzfeldt-Jakob* disease

- The disease was discovered in 1986 when a huge number of cows in England became ill
- Prion can be transmitted to people who **ingest** the brain or bone marrow of infected cattle (it is resistant to cooking)
- So far, about 100 people have died from this disease
- It was mainly present in younger people where the initial symptoms were **psychiatric** and then progressed to **neurological changes and dementia**
- Death occurred in an average of 14 months

Fatal familial insomnia

- It affects adults and ends in death after 1 to 2 years
- Hereditary prion disease, caused by a mutation in the *PrP* gene
- It is manifested by sleep attacks and disorders of the mostly autonomic nervous system (excessive tearing, hypertension, hyperventilation episodes, basal temperature disorder, dysarthria, diplopia, ataxia…)
- Infectious agent can be transmitted to experimental animals