"DNA Enveloped Viruses"

Human Herpes viruses

Herpesviridae is a large family of DNA viruses. The name comes from the Greek *'herpein'* to *'creep'* describing the chronic, latent or recurrent nature of infections. There are eight currently identified members of the human herpes virus belong to three families.

- **Alpha- Herpesviruses** (HSV-1, HSV-2 and VZV/HHV-3)
- **Beta- Herpesviruses** (CMV/ HHV-5, HH-6 and HHV-7)
- **Gamma- Herpesviruses** (EBV/HHV-4 and KSHV/HHV-8)

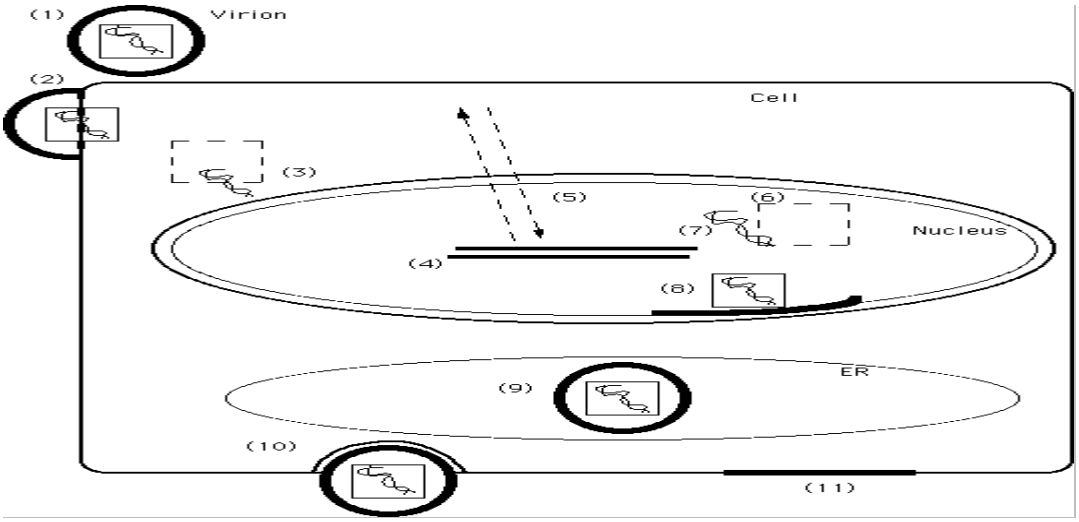
Viral Structure:

Herpesviruses all share a common structure, all Herpesviruses are composed of large double stranded linear **DNA genomes** encoding 100-200 genes encased within an **icosahedral protein** cage called capsid which is itself wrapped in a protein layer called the tegument containing both viral proteins and viral mRNA and a lipid bilayer membrane called the **envelope**, this whole particle is known as a **virion**. Replication in the nucleus and bud from nuclear membrane.

Herpesviruses replication:

The most extensive research on herpes viral replication has been done on HSV. It is believed that other Herpesviruses follow similar pathways with some at slower paces than others:

1. HSV virion attaches to host cell with the envelope glycoprotein onto heparan sulfate moieties of cellular proteoglycans, then it believed to bind to a secondary cellular receptor.
2. The viral envelope fuses to the plasma membrane in a pH- independent fashion such that the nucleocapsid enters the cytoplasm.
3. The capsid travels along the cytoskeleton to a nuclear pore where the viral DNA is released. The linear genome enters the nucleus and circularizes.
4. Once in the nucleus, the viral DNA is transcribed into mRNA by cellular RNA polymerase II.
5. After transcription in the nucleus all mRNA transcripts are translated into protein in the cytoplasm. Subsequently, the proteins can go to the nucleus, stay in the cytoplasm or become a part of the membrane bilayer.
6. Capsid proteins assemble in the nucleus to form empty capsids.
7. Full- length viral DNA is packaged to form nucleocapsid.
8. The nucleocapsid associate with segments of the nuclear membrane where tegument and glycosylated envelope proteins have bond. This association triggers envelopment by budding through the nuclear membrane.
9. Enveloped virions accumulate in the endoplasmic reticulum (ER).
10. Mature virions are released by exocytosis.
11. Virus- specific proteins are also found on the plasma membrane infected cells.



"A schematic diagram of herpesvirus replication"
***In HSV growth cycle proceeds rapidly requiring 8-16 hours for replication.**

Herpes simplex viruses

There are two distinct HSV: type 1 and type 2 (HSV-1, HSV-2). Their genomes are similar in organization and exhibit substantial sequence homology about 50-70%, the two viruses cross-react serologically. They differ in their mode of transmission; HSV-1 is spread by contact usually involving infected saliva, whereas HSV-2 is transmitted sexually or from a maternal genital infection to a newborn. This results in different clinical features of human infections.

Epidemiology:

HSV is spread by contact, as the virus is shed in saliva, tears, genital and other secretions, by far the most common form of infection results from a kiss given to a child or adult from a person shedding the virus. There are 2 peaks of incidence, the first at 0 - 5 years and the second in the late teens, when sexual activity commences. About 10% of the population acquires HSV infection through the genital route and the risk is concentrated in young adulthood.

Generally HSV-1 causes infection above the belt and HSV-2 below the belt. In fact, 40% of clinical isolates from genital sores are HSV-1, and 5% of strains isolated from the facial area are HSV-2. This data is complicated by oral sexual practices. Following primary infection, 45% of orally infected individuals and 60% of patients with genital herpes will experience recurrences.

Pathogenesis:

HSV is transmitted by contact of a susceptible person with an individual excreting virus. The virus must encounter mucosal surface or broken skin in order to infection initiated. HSV-1 is spread by respiratory droplets or saliva, HSV-2 is usually transmitted by genital routes. Viral replication occurs first at the site of infection, then virus invades local nerve endings and the virus establishes latency in the craniospinal ganglia. The exact mechanism of latency is not known, it may be true latency where there is no viral replication or viral persistence where there is a low level of viral replication. Reactivation - It is well known that many triggers can provoke a recurrence. These

include physical or psychological stress, infection; especially pneumococcal and meningococcal, fever, irradiation; including sunlight and menstruation. The virus follows axon back to the peripheral site and replication proceeds at the skin or mucosa membranes causing lesions then lesion which contain multinucleated giant cell found in the base of these lesions (ballooning of infected cell, production of nuclear inclusion bodies and margination of chromatin, cell fusion provides method for cell- to – cell spread of HSV. Many recurrences are asymptomatic.

HSV is involved in a variety of clinical manifestations which includes:-

1.Acute gingivostomatitis:

- Acute gingivostomatitis is the commonest manifestation of primary herpetic infection.
- The patient experiences pain and bleeding of the gums. Usually a self-limiting disease which lasts around 13 days.

2. Herpes Labialis (cold sore):

- Following primary infection, 45% of orally infected individuals will experience reactivation. The actual frequency of recurrences varies widely between individuals.
- Herpes labialis (cold sore) is a recurrence of oral HSV.
- A prodrome of tingling, warmth or itching at the site usually recurrence. About 12 hours later, redness appears followed by papules and then vesicles.

3. Ocular Herpes:

HSV causes a broad spectrum of ocular disease, ranging from mild superficial lesions involving the external eye, to severe sight-threatening diseases of the inner eye. Diseases caused include the following:-

- Primary HSV keratitis – dendritic ulcers
- Recurrent HSV keratitis
- HSV conjunctivitis
- Iridocyclitis, chorioretinitis and cataract

4. Herpes Genitalis:

- Many sites can be involved which includes the penis, vagina, cervix, anus, vulva, bladder, the sacral nerve routes, the spinal and the meninges. The lesions of genital herpes are particularly prone to secondary bacterial infection eg. S.aureus, Streptococcus, Trichomonas and Candida Albicans.
- Dysuria is a common complaint, in severe cases, there may be urinary retention.
- Local sensory nerves may be involved leading to the development of a radiculitis. A mild meningitis may be present.
- 60% of patients with genital herpes will experience recurrences.

5. Encephalitis and Neonatal herpes:

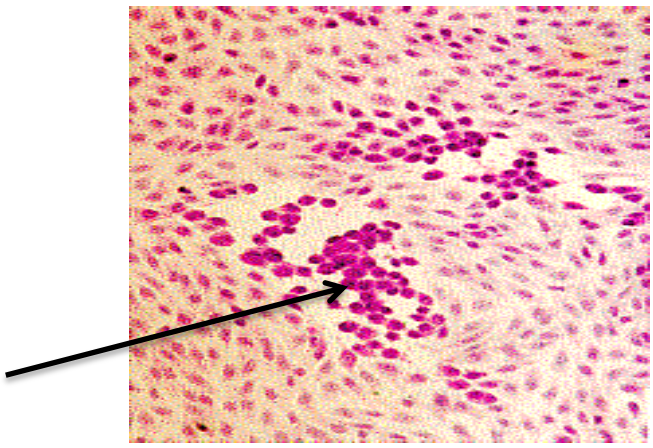
- Herpes Simplex encephalitis is one of the most serious complications of herpes simplex disease. There are two forms:
- Neonatal – there is global involvement and the brain is almost liquefied. The mortality rate approaches 100%.
- Focal disease – the temporal lobe is most commonly affected. This form of the disease appears in children and adults. It is possible that many of these cases arise from reactivation of virus. The mortality rate is high (70%) without treatment.

Laboratory Diagnosis:

- **Direct Detection:**

  1.Cytopathology and electron microscopy of scraping obtained from the base of vesicle rapid result but cannot distinguish between HSV and VZV

  2.Immunofluorescence of skin scrappings - can distinguish between HSV and VZV.



Cytopathic Effect of HSV in cell culture: Note the ballooning of cells.

- **Virus Isolation:**

  HSV-1 and HSV-2 are among the easiest viruses to cultivate. It usually takes only 1 - 5 days for a result to be available.

- **Serology:**

  Not that useful in the acute phase because it takes 1-2 weeks for before antibodies appear after infection. Used to document to recent infection.

- **Molecular technique:**

  PCR assays can be used to detect virus and are sensitive and specific.

Treatment:

Generally, antiviral chemotherapy is indicated where the primary infection is especially severe, where there is dissemination, where sight is threatened, and herpes simplex encephalitis. Several antiviral drugs have proved effective against HSV infections including acyclovir, valacyclovir and vidarabine. All inhibitors of viral DNA synthesis.

Varicella-Zoster Virus (VZV):

Varicella (**chickenpox**) is a mild highly contagious disease of children characterized clinically by A generalized vesicular eruption of the skin and mucosa membranes. **Zoster** (**shingles**) is a sporadic incapaciting disease of adults or immunocompromised individuals that is characterized by a rash limited in distribution of the skin innervated by a single sensory ganglion. Both diseases are caused by the same virus. Varicella is the acute disease that follows primary contact with the virus, whereas zoster is the response of the immune host to reactivation of varicella virus in latent form in neurons in sensory ganglia, VZV has the following properties:

- Varicella-zoster virus is morphologically identical to HSV
- Belong to the alphaherpesvirus subfamily of Herpesviruses
- Double stranded DNA enveloped virus
- One antigenic serotype only, although there is some cross reaction with HSV.

Epidemiology:

VSV occur worldwide. Varicella is highly communicable, with an attack rate of 90% in close contacts. Varicella is one of the classic diseases of childhood, with the highest prevalence occurring in the 4 - 10 years old age group. Herpes zoster, in contrast, occurs sporadically and evenly throughout the year. It is much more common in winter and spring than in summer in temperate climates. Adults will experience at least one zoster attack during their lifetime after the age of 50.

Pathogenesis:

The route of infection is the mucosa of the upper respiratory tract or the conjunctiva. Following initial replication in regional lymph nodes, primary viremia spreads virus leads to replication in the liver and spleen. Secondary viremia involving infected mononuclear cells transported virus to the skin where the typical rash develops. Swelling of epithelial cells, ballooning degeneration and vesicle formation. The skin lesions of zoster are histopathologically identical to those of varicella there is also an acute inflammation of the sensory nerves and ganglia the distribution of lesions in the skin corresponds closely to the areas of innervation from an individual dorsal root ganglion.
VZV is involved in the following clinical manifestations:

Varicella:

- Primary infection results in varicella (chickenpox)
- Incubation period of 14-21 days
- Presents fever, lymphadadenopathy. a widespread vesicular rash. The rash first appear in the trunk and then on the face, the limbs and mucosa of mouth.
- Complications are rare involve secondary bacterial infection.

Zoster:

- It is occasionally developed in healthy young adults and usually in immunocompromised persons as a result of disease, therapy or aging. The vast majority of patients are more than 50 years of age.

- It usually starts with severe pain in the area of skin or mucosa supplied by one or more groups of sensory nerves and ganglia. Within a few days after onset a crop of vesicles appears over the skin supplied by the effected nerves. The trunk, head, neck are most affected with ophthalmic division in 10-15% of cases.
- Complications are rare and include encephalitis.

Immunity:
Previous infection with varicella confers lifelong immunity, but zoster can occur with advancing age.

Laboratory Diagnosis:

The clinical presentations of varicella or zoster are so characteristic that laboratory confirmation is rarely required. Laboratory diagnosis is required only for atypical presentations, particularly in the immunocompromised.

- Virus Isolation - rarely carried out as it requires 2-3 weeks for a result.
- Direct detection - electron microscopy may be used for vesicle fluids (Tzanck smear) but cannot distinguish between HSV and VZV. Immunofluorescence on skin scrapings can distinguish between the two.
- Serology - the presence of VZV IgG is indicative of past infection and immunity. The presence of IgM is indicative of recent primary infection.

Treatment:
Gamma- globulin of high varicella-zoster virus antibody titer can be used to prevent the development of the illness in patients exposed to varicella who are at high risk of developing sever disease. Three drugs can be used for the treatment of herpes zoster: acyclovir, valicyclovir, and famciclovir. There appears to be little difference in efficacy between them.

Cytomegalovirus (CMV):

Cytomegaloviruses are the agents of the most common congenital infection. Cytomegalovirus has the largest genome of the human Herpesviruses. The name for the classic cytomegalic inclusion disease derives from the propensity for massive enlargement of cytomegalic infected cells. The disease is an infectious mononucleosis like syndrome.

Epidemiology:
Cytomegalovirus is endemic in all parts of the world. It is present throughout the year with no seasonal variation seen in infection rates. CMV is one of the most successful human pathogens; it can be transmitted vertically or horizontally. Transmission may occur in utero, perinatally or postnatally. Once infected, the person carries the virus for life which may be activated from time to time, during which infectious virions appear in the urine and the saliva. Reactivation can also lead to vertical transmission.

Pathogenesis:

Cytomegalovirus may be transmitted person-to-person in several different ways; all requiring close contact with virus bearing material, there is a 4-8 weeks incubation period in normal older children and adults after viral exposure. Once infected, the virus remains in the person for life and may be reactivated from time to time, especially in immunocompromised individuals. The virus may be transmitted in utero, perinatally, or postnatally. Perinatal infection is acquired mainly through infected genital secretions, or breast milk. Overall, 2 - 10% of infants are infected by the age of 6 months worldwide. Perinatal infection is thought to be 10 times more common than congenital infection. Postnatal infection mainly occurs through saliva. Sexual transmission may occur as well as through blood and blood products and transplanted organ.

Like all Herpesviruses, cytomegalovirus establishes lifelong latent infections; virus can be shed intermittently from the pharynx and in the urine for months to years after primary infection. Prolonged cytomegalovirus infection of the kidney does not seem to be deleterious in normal persons, salivary gland involvement is common and chronic. Primary cytomegalovirus infections in immunosuppressed hosts are much more severe than in normal hosts.

Clinical manifestations:

- Congenital infection - may result in cytomegalic inclusion disease. Defined as the isolation of CMV from the saliva or urine within 3 weeks of birth. May be transmitted to the fetus during all stages of pregnancy (primary or reactivated infection during pregnancy). No evidence of teratogenicity, damage to the fetus results from destruction of target cells once they are formed.
- Perinatal infection - usually asymptomatic, because the babies have maternal- transmitted CMV IgG antibody. It is result from exposure to infected secretions of maternal genital tract at delivery or breast milk that contains the virus.
- Postnatal infection - usually asymptomatic. However, in a minority of cases, the syndrome of infectious mononucleosis may develop which consists of fever, lymphadenopathy, and splenomegaly. The heterophil antibody test is negative although atypical lymphocytes may be found in the blood.
- Immunocompromised patients such as transplant recipients and AIDS patients are prone to severe CMV disease such as pneumonitis, retinitis, colitis, and encephalopathy.
- Reactivation or reinfection with CMV is usually asymptomatic except in immunocompromised patients.

Laboratory Diagnosis:

- **Direct detection**
  1- Biopsy specimens may be examined histologically for CMV inclusion antibodies or for the presence of CMV antigens. However, the sensitivity may be low.
  2- The pp65 CMV antigenaemia test is now routinely used for the rapid diagnosis of CMV infection in immunocompromised patients.

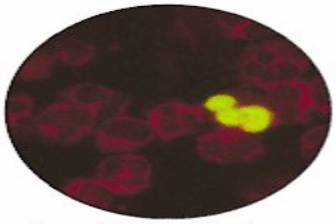


Figure 4 CMV pp65 antigens detected in nuclei of peripheral blood neutrophils

- **Virus Isolation:**
  1- Conventional cell culture is regarded as gold standard but requires up to 4 weeks for result.
  2- More useful are rapid culture methods such as the DEAFF test which can provide a result in 24-48 hours.
- **Serology:**
  1- The presence of CMV IgG antibody indicates past infection.
  2- The detection of IgM is indicative of primary infection although it may also be found in immunocompromised patients with reactivation.
- **PCR technique.**

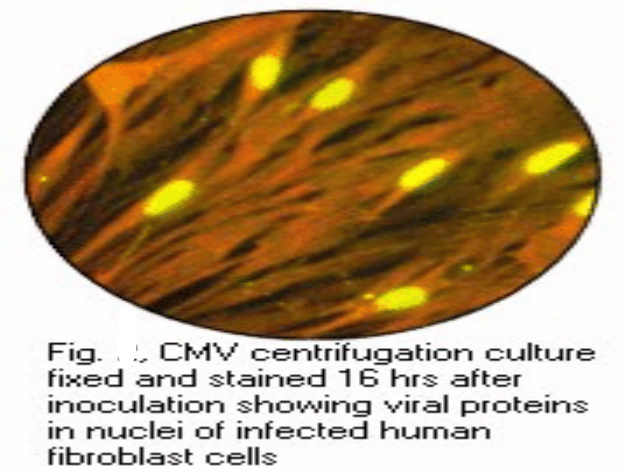


Fig. ٤: CMV centrifugation culture fixed and stained 16 hrs after inoculation showing viral proteins in nuclei of infected human fibroblast cells

"DEAFF test for CMV"

"Table show Specimens used for Laboratory Diagnosis of CMV"

	Site of virus culture				Serology	
	Urine	Saliva	Blood	Tissue	IgG	IgM
Neonates	+	+	-	-	-	+
Adults	+	-	+	-	+	+
Pregnant women	-	-	-	-	+	+
Immunocompromised	+	+	+	+	-	-

Medical Care:

The drug of choice for treatment of CMV disease is intravenous Ganciclovir for cytomegalovirus retinitis, bone marrow allograft recipients and renal transplant patients, although Valacyclovir may be used for nonsevere CMV treatment in selected cases. Valacyclovir is a prodrug of Ganciclovir that is activated in the gut and liver to Ganciclovir. Valacyclovir 900 mg orally once daily is equivalent to once- daily intravenous Ganciclovir 5 mg/kg.

Epstein - Barr virus (EBV):

EBV is a ubiquitous gammaherpesvirus that is the causative agent of acute infectious mononucleosis and is associated with nasopharyngeal carcinoma, Burkitt lymphoma, Hodgkin and non-Hodgkin lymphomas, other lymphoproliferative disorders in immunodeficient individuals and gastric carcinoma. Genome is a linear double stranded DNA molecule does not normally integrate into the cellular DNA but forms circular episomes which reside in the nucleus. Membrane is derived by budding of immature particles through cell membrane and is required for infectivity.

Epidemiology:

The virus is transmitted by contact with saliva, in particularly through kissing. Two epidemiological patterns are seen with EBV. In developed countries, 2 peaks of infection are seen: the first in very young preschool children aged 1 - 6 and the second in adolescents and young adults aged 14 - 20 eventually 80-90% of adults are infected. In developing countries, infection occurs at a much earlier age so that by the age of two, 90% of children are seropositive.

Pathogenesis:

EBV is commonly transmitted by infected saliva and initiates infection in the oropharynx, viral replication occur in the epithelial cells (or surface B lymphocytes) of the pharynx and salivary glands. Infected B cell spread the infection from the oropharynx throughout the body. In normal individuals most virus infected cells are eliminated but small numbers of latently infected lymphocytes persist for the lifetime of the host (one in 10^5- 10^6 B cells).

Primary infection in children is usually subclinical but if they occur in young adults acute infectious mononucleosis often develops. Mononucleosis is a polyclonal stimulation of lymphocytes. Reactivation of EBV latent infections can occur in immunosuppression as evidenced by increased level of virus in saliva and DNA in blood cells.

Disease Association:

1. Infectious Mononucleosis
2. Burkitt's lymphoma
3. Nasopharyngeal carcinoma
4. Lymphoproliferative disease and lymphoma in the immunosuppressed.
5. X-linked lymphoproliferative syndrome
6. Chronic infectious mononucleosis
7. Oral leukoplakia in AIDS patients
8. Chronic interstitial pneumonitis in AIDS patients.

Infectious Mononucleosis: after an incubation period of 30-50 days, symptoms of headache, fever, malaise, fatigue and sore throat occur. Enlarged lymph nodes and spleen are characteristics. Some

patients develop signs of hepatitis. The typical illness is self-limited and lasts 2-4 weeks during this disease there is an increase in the number of circulating white blood cells with a predominance of lymphocytes (large atypical lymphocytes).

Burkitt's lymphoma: it is a tumor of the jaw in African children (usually occurs in children aged 3-14 years) and young adults. It is restricted to areas with holoendemic malaria. Therefore it appears that malaria infection is a cofactor. BL cells show a reciprocal translocation between the long arm of chromosome 8 and chromosomes 14, 2 or 22, this translocation result in the c-myc oncogene being transferred to the Immunoglobulin gene regions. This results in the deregulation of the c-myc gene. It is thought that this translocation is probably already present by the time of EBV infection and is not caused by EBV.

Nasopharyngeal carcinoma: Nasopharyngeal carcinoma (NPC) is a malignant tumor of the squamous epithelium of the nasopharynx. It is very prevalent in China, where it is the commonest tumor in men. Multiple copies of EBV genome and EBV EBNA-1 antigen can be found in cells of undifferentiated NPC. Patients with NPC have high titres of antibodies against various EBV antigens.

Laboratory Diagnosis:

- Acute EBV infection is usually made by the heterophil antibody test and/or detection of anti-EBV VCA IgM.
- Cases of Burkitt's lymphoma should be diagnosed by histology. The tumor can be stained with antibodies to lambda light chains which should reveal a monoclonal tumor of B-cell origin. In over 90% of cases, the cells express IgM at the cell surface.
- Cases of NPC should be diagnosed by histology.
- The determination of the titre of anti-EBV VCA IgA in screening for early lesions of NPC and also for monitoring treatment.
- Nucleic acid hybridization is the most sensitive means of detecting EBV in patients materials, EBV can be isolated from saliva, peripheral blood or lymphoid tissue by immortalization of normal human lymphocytes usually obtained from umbilical cord blood.

Medical Care:

- A vaccine against EBV which prevents primary EBV infection should be able to control both BL and NPC.
- Acyclovir reduces EBV shedding from the oropharynx but it does not affect the number of EBV-immortalized B- cell, has no effect on the symptoms of mononucleosis and is no proved benefit in the treatment of EBV- associated lymphomas in immunocompromised patients.

Poxviruses

Poxviruses are the largest and most complex of viruses, poxviruses are divided into two subfamilies based on whether they infect vertebrates or not. There are three viruses causing disease in human:

1. **Viriola:** (smallpox) now eliminated
2. **Vaccinia:** (localized lesion used for smallpox vaccination)
3. **Molluscum contagiosum:** (benign skin nodules)

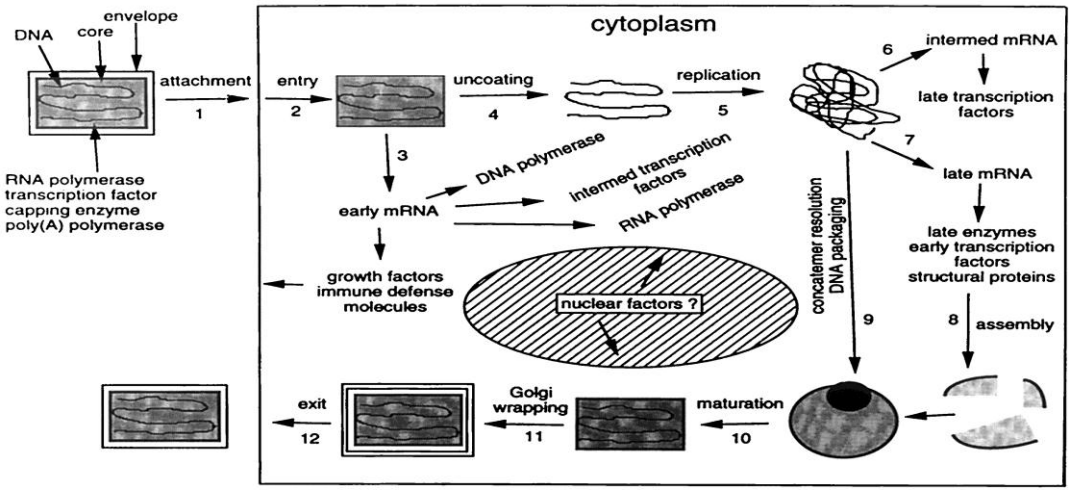
Important properties:

- Complex structure, oval or brick-shaped with external surface shows ridges
- Genome is double-stranded DNA linear with terminal loops
- About envelope the virion assembly involves formation of multiple membranes
- Replication is cytoplasmic factories

Replication cycle of Pox virus:

Poxviruses are generally containing a single, linear, double- stranded DNA genome, replication occur in the cytoplasm of the cell. The virus Contain DNA – dependant RNA pohymerase within particle which help the viral replication in cytoplasm, the virus not need to cellular RNA polymerase. Replication cycle involves the following steps:

1. **Entry:** intracellular mature virion (IMV) particles bind to unknown receptors and fuse with the cell membrane. Extracellular enveloped virion (EEV) particles bind to unknown receptors and endocytosed into the cell.
2. **Initial Uncoating:** the viral core particle (CORE) containing the viral genome, the viral DNA-dependent RNA polymerase and other enzymes released into the cytoplasm.
3. **Early Transcription:** Early genes are transcribed and translated immediately upon core particle entry into the cytoplasm of the cell.
4. **Translocation:** the viral core particle translocate to the outside of the cell nucleus.
5. **Secondary Uncoating:** the viral nucleoprotein (NP) complex which contains viral genome is released, at this point the viral genome is replicated as a concatemer and transcription and translation of intermediated genes.
6. **Late Transcription:** the viral late genes are transcribed and translated.
7. **Assembly:** concatemeric intermediates are resolved into linear double- stranded DNA and packaged with late viral proteins into immature virions (IV).
8. **Release:** IVs mature into IMVs via processing of IV through the Golgi apparatus. The IMVs are transported to the periphery of the cell and release by cell lysis or picking up from the cell plasma membrane.



"A schematic represented replication cycle of Pox viruses"

Pathogenesis and Clinical Manifestation:

Incubation period 7-14 days. The portal of entry was the mucosa membrane of the upper respiratory tract. After viral entry the following events take place: (1) primary multiplication in the lymphoid tissue draining the site of entry; (2) transient viremia and infection of reticuloendothelial cells throughout the body; (3) a secondary phase of multiplication in those cells leading to (4) a secondary more intensive viremia (5) the clinical disease. By the sixth to ninth day lesion in the mouth tended to ulcerate and discharge virus, virus originated in lesions in the mouth and upper respiratory tract. Later pustules broke down and discharged virus into the environment of the smallpox patient. Crust prolonged 2-3 weeks. Immunity is long- life.

Laboratory Diagnosis:

1. Isolation of virus: skin lesions are the specimen of choice for viral detection and isolation.
2. Serology: antibodies appear after the first week of infection that can be detect by HI, ELISA, RIA and IF tests.

Medical Care:

- Vaccination with vaccine**:** vaccinating between 1 and 2 years of age and revaccination at 3 year intervals.
- Rifampicin inhibits viral DNA dependent RNA polymerase.