Viral production, Replication:-

Viruses multiply only in living cells. The host cell must provide the energy and synthetic machinery and the low molecular-weight precursors for the synthesis of viral proteins and nucleic acids.

The virus replication occurs in seven stages, namely;

1. Attachment
2. Entry,
3. Uncoating,
4. Transcription / mRNA production,
5. Synthesis of virus components,
6. Virion assembly and
7. Release (Liberation Stage).

Attachment is a specific binding between viral capsid proteins and specific receptors on the host cellular surface , It is the first step of viral replication. The virus attaches to the cell membrane of the host cell. It then injects its DNA or RNA into the host to initiate infection. In animal cells these viruses get into the cell through the process of endocytosis which works through fusing of the virus and fusing of the viral envelope with the cell membrane of the animal cell and in plant cell it enters through the process of pinocytosis which works on pinching of the viruses.

Penetration or **viral entry** follows attachment: Virions enter the host cell through receptor-mediated **endocytosis or membrane fusion**. This occurs by one or more processes.

- Enveloped viruses fuse their envelope with the membrane of the host cell. This involves local digestion of the viral and cellular membranes, fusion of the membranes and concomitant release of the nucleocapsid into the cytoplasm.

- Naked viruses bind to receptor sites on the cellular membrane, digest the membrane and enter into the cytoplasm intact.

- Both naked and enveloped viruses can be ingested by phagocytic cells. However, in this process they enter the cytoplasm enclosed in a cytoplasmic membrane derived from the phagocytic cell.

Uncoating

Uncoating is a process in which the viral capsid is removed: This may be by degradation by viral enzymes or host enzymes or by simple dissociation; the end-result is the releasing of the viral genomic nucleic acid**.**

Transcription / mRNA production

Replication of viruses involves primarily multiplication of the genome. Replication involves synthesis of viral messenger RNA (mRNA) from "early" genes (with exceptions for positive sense RNA viruses), viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: "late" gene expression is, in general, of structural or virion proteins.

For some RNA viruses, the infecting RNA produces messenger RNA (mRNA). This is translation of the genome into protein products. For

others with negative stranded RNA and DNA, viruses are produced by transcription then translation.

The mRNA is used to instruct the host cell to make virus components. The virus takes advantage of the existing cell structures to replicate itself.

Synthesis of virus components

The following components are manufactured by the virus using the host's existing organelles:

- Viral proteins: Viral mRNA is translated on cellular ribosomes into two types of viral protein:
  - Structural: proteins which make up the virus particle
  - Nonstructural: proteins not found in the virus particle, mainly enzymes for virus genome replication
- Viral nucleic acid (genome replication): New viral genomes are synthesized; templates are either the parental genome or newly formed complementary strands, in the case of single-stranded genomes. These genomes are made by either a viral polymerase or (in some DNA viruses) a cellular enzyme, particularly in rapidly dividing cells.

Virion assembly

A virion is simply an active or intact virus particle. In this stage, newly synthesized genome (nucleic acid), and proteins are assembled to form new virus particles.

This may take place in the cell's nucleus, cytoplasm, or at plasma membrane for most developed viruses.

Release (liberation stage)

Viruses can be released from the host cell by lysis, a process that kills the cell by bursting its membrane . Some viruses undergo a **lysogenic** cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome. The viral genome is then known as a "provirus" or, in the case of bacteriophages a "prophage". Whenever the host divides, the viral genome is also replicated. The viral genome is mostly silent within the host. At some point, the provirus or prophage may give rise to active virus, which may lyse the host cells. Enveloped viruses (e.g., HIV) typically are released from the host cell by budding. During this process the virus acquires its envelope, which is a modified piece of the host's plasma or other, internal membrane.

The viruses, now being mature are released by either sudden rupture of the cell, or gradual extrusion (force out) of enveloped viruses through the cell membrane.

The new viruses may invade or attack other cells, or remain dormant in the cell. In the case of bacterial viruses, the release of progeny virions takes place by lysis of the infected bacterium. However, in the case of animal viruses, release usually occurs without cell lysis.

Effects of viruses on host cell

Viruses depend on the host cells that they infect to reproduce. When found outside of host cells, viruses exist as a protein coat or capsid, sometimes enclosed within a membrane. The capsid encloses either DNA or RNA which codes for the virus elements. While in this form outside the cell, the virus is metabollically inert; When it comes into contact with a host cell, a virus can insert its genetic material into its host, literally

taking over the host's functions. An infected cell produces more viral protein and genetic material instead of its usual products. Some viruses may remain **dormant** inside host cells for long periods, causing no obvious change in their host cells (a stage known as the **lysogenic** phase). But when a dormant virus is stimulated, it enters the **lytic** phase: new viruses are formed, self-assemble, and burst out of the host cell, killing the cell and going on to infect other cells. Viruses are species specific, but almost every species on Earth can be affected by some form of virus.

- ✓ **The lytic cycle** involves the reproduction of viruses using a host cell to manufacture more viruses; the viruses then burst out of the cell. With lytic phages, bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, the phage progeny can find new hosts to infect. An example of a lytic bacteriophage is T4, which infects E. coli found in the human intestinal tract. Lytic phages are more suitable for phage therapy.

  Bacteriophages are viruses that infect bacteria. Bacteriophages may have a lytic cycle or a lysogenic cycle, and a few viruses are capable of carrying out both. When infection of a cell by a bacteriophage results in the production of new virions, the infection is said to be productive.

- ✓ **The lysogenic cycle** involves the incorporation of the viral genome into the host cell genome, infecting it from within, The lysogenic cycle does not result in immediate lysing of the host cell. Their viral genome will integrate with host DNA and replicate along with it fairly harmlessly, or may even become established as a plasmid. The virus remains dormant until host conditions deteriorate, perhaps due to depletion of nutrients; then, the endogenous phages (known as prophages) become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycle allows

the host cell to continue to survive and reproduce, the virus is reproduced in all of the cell's offspring. An example of a bacteriophage known to follow the lysogenic cycle and the lytic cycle is the phage lambda of *E. coli*.

Viral pathogenesis

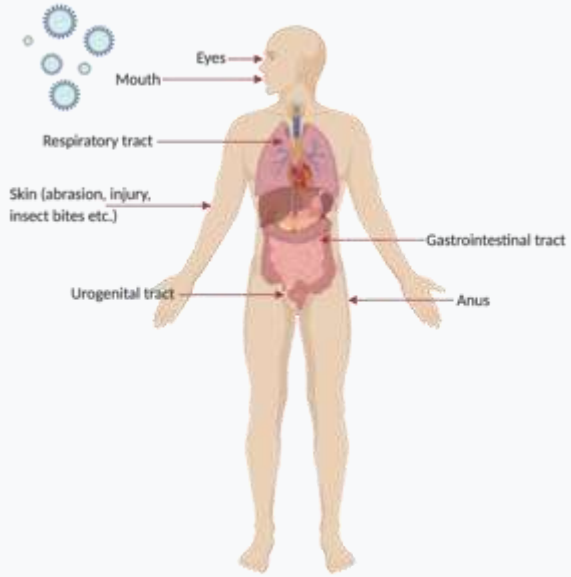
Viral pathogenesis is the study of the process and mechanisms by which viruses cause diseases in their target hosts, often at the cellular or molecular level. It is a specialized field of study in virology.

Pathogenesis is a qualitative description of the process by which an initial infection causes disease. Viral disease is the sum of the effects of viral replication on the host and the host's subsequent immune response against the virus. Viruses are able to initiate infection, disperse throughout the body, and replicate due to specific virulence factors.

There are several factors that affect pathogenesis. Some of these factors include virulence characteristics of the virus that is infecting. In order to cause disease, the virus must also overcome several inhibitory effects present in the host. Some of the inhibitory effects include distance, physical barriers and host defenses. These inhibitory effects may differ among individuals due to the inhibitory effects being genetically controlled.

Viral pathogenesis is affected by various factors: (1) transmission, entry and spread within the host, **(2)** tropism, this response is dependent on the direction of the stimulus (as opposed to nastic movements which are non-directional responses). Viruses and other pathogens also affect what is called "host tropism", "tissue tropism", or "cell tropism"; in which case tropism refers to the way in which different viruses/pathogens have

evolved to preferentially target specific host species, specific tissue, or specific cell types within those species. **(3)** virus virulence and disease mechanisms, **(4)** host factors and host defense.



Typical sites of virus entry into the body: The first steps of viral infection is determined by the site at which the virus implants into the body. This would subsequently dictate the mechanisms of viral pathogenesis.

Enveloped and Nonenveloped viruses

A **viral envelope** is the outermost layer of many types of viruses.

It protects the genetic material in their life-cycle when traveling between host cells.

Not all viruses have envelopes.

The envelopes are typically derived from portions of the host cell membranes (phospholipids and proteins), but include some viral glycoproteins. They may help viruses avoid the host immune system. Glycoproteins on the surface of the envelope serve to identify and bind to receptor sites on the host's membrane. The viral envelope then fuses with the host's membrane, allowing the capsid and viral genome to enter and infect the host.

All enveloped viruses also have a capsid, another protein layer, between the envelope and the genome.

Examples of enveloped viruses

Several classes of enveloped viruses that contain human pathogens are recognized.

- DNA enveloped viruses
  - Herpesviruses
  - Poxviruses
  - Hepadnaviruses

- DNA Nonenveloped viruses
- Adenoviridae
- Papillomaviridae

- RNA enveloped viruses
  - Coronavirus
  - Hepatitis D
  - Orthomyxovirus
  - Paramyxovirus
  - Retroviruses
- RNA Nonenveloped viruses
  - Picornaviridae

❖ DNA enveloped viruses

1- HERPES VIRUSES

The herpesvirus family contains six important human pathogens: herpes simplex virus types 1 and 2, varicella-zoster virus, cytomegalovirus, Epstein–Barr virus, and human herpesvirus 8 (the cause of Kaposi's sarcoma).

Important Properties

All herpesviruses are structurally similar. Each has an **icosahedral** core surrounded by a lipoprotein **envelope** . The genome is linear double-stranded DNA. The virion does not contain a polymerase.

They replicate in the nucleus, form intranuclear inclusions, and are the only viruses that obtain their envelope by budding from the nuclear membrane. The virions of herpesviruses possess a **tegument** located between the nucleocapsid and the envelope. This structure contains regulatory proteins, such as transcription and translation factors, which play a role in viral replication.

Herpesviruses are noted for their ability to cause **latent infections**. In these infections, the acute disease is followed by an asymptomatic period during which the virus remains in a quiescent (latent) state. When the patient is exposed to an inciting agent or immunosuppression occurs, reactivation of virus replication and disease can occur.

TABLE : Important Features of Common Herpesvirus Infections

Virus	Primary Infection	Usual Site of Latency	Recurrent Infection
HSV-1	Gingivostomatitis[1]	Cranial sensory ganglia	Herpes labialis,[2,3] ence litis, keratitis
HSV-2	Herpes genitalis, perinatal disseminated disease	Lumbar or sacral sensory ganglia	Herpes genitalis[2,3]
VZV	Varicella	Cranial or thoracic sensory ganglia	Zoster[2]
EBV	Infectious mononucleosis[1]	B lymphocytes	Asymptomatic sheddi
CMV	Congenital infection (in utero), mononucleosis[1]	Monocytes	Asymptomatic sheddi
HHV-8[5]	Uncertain[6]	Uncertain	Kaposi's sarcoma

CMV = cytomegalovirus; EBV = Epstein–Barr virus; HHV-8 = human herpesvirus 8; HSV = herpes simplex virus; VZV = varice

[1]Primary infection is often asymptomatic.

[2]In immunocompromised patients, dissemination of virus can cause life-threatening disease.

[3]Asymptomatic shedding also occurs.

[4]Latent EBV infection predisposes to B-cell lymphomas.

[5]Also known as Kaposi's sarcoma–associated herpesvirus.

[6]A mononucleosis-like syndrome has been described. Kaposi's sarcoma itself also can result from a primary infection.

Some information is available regarding the mechanism by which herpes simplex virus (HSV) and cytomegalovirus (CMV) initiate and maintain the latent state. Shortly after HSV infects neurons, a set of "**latency-associated transcripts**" (LATS) are synthesized. **Three of the herpesviruses, HSV types 1 and 2 and varicella-zoster virus (VZV)**, cause a **vesicular rash,** both in primary infections and in reactivations. Primary infections are usually more severe than reactivations. The other two herpesviruses, CMV and Epstein–Barr virus (EBV), do not cause a vesicular rash.

Four herpesviruses, namely HSV types 1 and 2, VZV, and CMV, induce the formation of **multinucleated giant cells,** which can be seen microscopically in the lesions. The importance of giant cells is best illustrated by the Tzanck smear, which reveals multinucleated giant cells in a smear taken from the painful vesicles of the genitals caused by HSV type 2 .

The herpesvirus family can be subdivided into three categories based on the type of cell most often infected and the site of latency. The alpha herpesviruses, consisting of HSV types 1 and 2 and VZV, infect epithelial cells primarily and cause latent infection in neurons. The beta herpesviruses, consisting of CMVs and human herpesvirus 6, infect and become latent in a variety of tissues. The gamma herpesviruses, consisting of EBV and human herpesvirus 8 (HHV-8, Kaposi's sarcoma–associated virus), infect and become latent primarily in lymphoid cells.

Certain herpesviruses are associated with or cause cancer in humans (e.g., Epstein–Barr virus is associated with Burkitt's lymphoma and nasopharyngeal carcinoma, and human herpesvirus 8 causes Kaposi's sarcoma). Several herpesviruses cause cancer in animals (e.g., leukemia in monkeys and lymphomatosis in chickens).

Herpes Simplex Viruses (HSV)

HSV type 1 (HSV-1) and type 2 (HSV-2) are distinguished by two main criteria: antigenicity and location of lesions. Lesions caused by HSV-1 are, in general, above the waist, whereas those caused by HSV-2 are below the waist.

Diseases

HSV-1 causes acute gingivostomatitis, recurrent herpes labialis (cold sores), keratoconjunctivitis (keratitis), and encephalitis, primarily in adults.

HSV-2 causes herpes genitalis (genital herpes), neonatal encephalitis and other forms of neonatal herpes, and aseptic meningitis.

Infection by HSV-1 or HSV-2 is a common cause of erythema multiforme.

Transmission & Epidemiology

HSV-1 is transmitted primarily in **saliva,** whereas HSV-2 is transmitted by **sexual contact**. As a result, HSV-1 infections occur mainly on the face, whereas HSV-2 lesions occur in the genital area. However, oral–genital sexual practices can result in HSV-1 infections of the genitals and HSV-2 lesions in the oral cavity (this occurs in about 10%–20% of cases). Although transmission occurs most often when active lesions are present, asymptomatic shedding of both HSV-1 and HSV-2 does occur and plays an important role in transmission.

The number of HSV-2 infections has markedly increased in recent years, whereas that of HSV-1 infections has not. Roughly 80% of people

in the United States are infected with HSV-1, and 40% have recurrent herpes labialis. Most primary infections by HSV-1 occur in childhood, as evidenced by the early appearance of antibody. In contrast, antibody to HSV-2 does not appear until the age of sexual activity.

Pathogenesis & Immunity

The virus replicates in the skin or mucous membrane at the initial site of infection, and then migrates up the neuron by retrograde axonal flow and becomes **latent in the sensory ganglion cells**. In general, **HSV-1** becomes latent in the **trigeminal ganglia.**

whereas **HSV-2** becomes latent in the **lumbar** and **sacral ganglia**. During latency, most—if not all—viral DNA is located in the cytoplasm rather than integrated into nuclear DNA. The virus can be reactivated from the latent state by a variety of inducers (e.g., sunlight, hormonal changes, trauma, stress, and fever), at which time it migrates down the neuron and replicates in the skin, causing lesions.

The typical skin lesion is a **vesicle** that contains serous fluid filled with virus particles and cell debris. When the vesicle ruptures, virus is liberated and can be transmitted to other individuals. **Multinucleated giant cells** are typically found at the base of herpesvirus lesions.

Immunity is type-specific, but some cross-protection exists. However, immunity is incomplete, and both reinfection and reactivation occur in the presence of circulating IgG. **Cell-mediated immunity** is important in limiting herpesviruses, because its suppression often results in reactivation, spread, and severe disease.

Clinical Findings

HSV-1 causes several forms of primary and recurrent disease:

(1) **Gingivostomatitis** occurs primarily in children and is characterized by fever, irritability, and vesicular lesions in the mouth. The primary disease is more severe and lasts longer than recurrences. The lesions heal spontaneously in 2 to 3 weeks. Many children have asymptomatic primary infections.

(2) **Herpes labialis** (fever blisters or cold sores) is the milder, recurrent form and is characterized by crops of vesicles, usually at the mucocutaneous junction of the lips or nose (Figure). Recurrences frequently reappear at the same site.

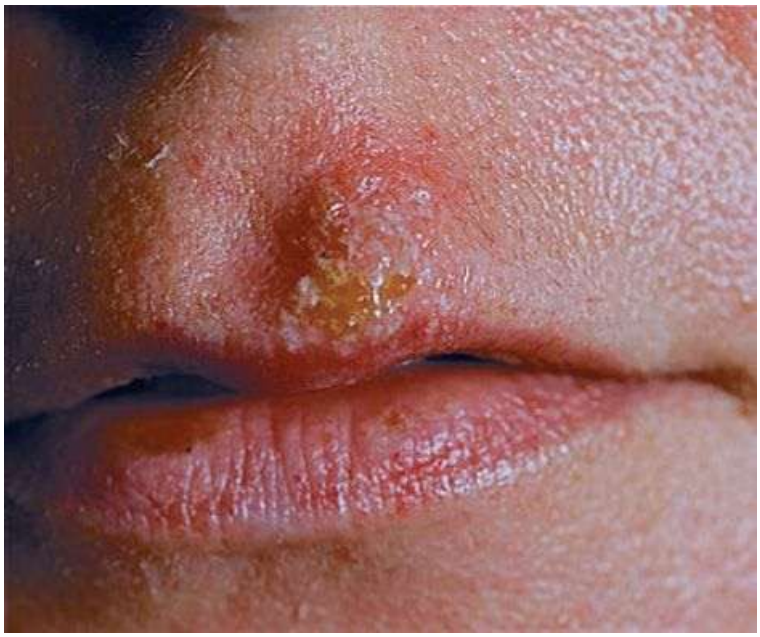


FIGURE - Herpes labialis—note vesicles on upper lip adjacent to the vermillion border of the lip caused by herpes simplex virus type 1. (Figure courtesy of Jack Resneck, Sr., MD.)

(3) **Keratoconjunctivitis** is characterized by corneal ulcers and lesions of the conjunctival epithelium. Recurrences can lead to scarring and blindness.

(4) **Encephalitis** caused by HSV-1 is characterized by a necrotic lesion in one temporal lobe. Fever, headache, vomiting, seizures, and altered mental status are typical clinical features. The onset may be acute or

protracted over several days. The disease occurs as a result of either a primary infection or a recurrence.

Magnetic resonance imaging often reveals the lesion. Examination of the spinal fluid typically shows a moderate increase of lymphocytes, a moderate elevation in the amount of protein, and a normal amount of glucose. HSV-1 encephalitis has a high mortality rate and causes severe neurologic sequelae in those who survive.

HSV-2 causes several diseases, both primary and recurrent:

(1) **Genital herpes** is characterized by painful vesicular lesions of the male and female genitals and anal area. The lesions are more severe and protracted in primary disease than in recurrences. Primary infections are associated with fever and inguinal adenopathy. Asymptomatic infections occur in both men (in the prostate or urethra) and women (in the cervix) and can be a source of infection of other individuals. Many infections are asymptomatic (i.e., many people have antibody to HSV-2 but have no history of disease).

Approximately 80% to 90% of herpes genitalis cases are caused by HSV-2. The remainder are caused by HSV-1 as a result of oral–genital contact. The clinical importance of this is that suppressive chemoprophylaxis for HSV-2 lesions should be considered because lesions caused by HSV-2 are more likely to recur than lesions caused by HSV-1.

(2) **Neonatal herpes** originates chiefly from contact with vesicular lesions within the birth canal. In some cases, although there are no visible lesions, HSV-2 is shed into the birth canal (asymptomatic shedding) and can infect the child during birth. Neonatal herpes varies from severe disease (e.g., disseminated lesions or encephalitis) to milder local lesions (skin, eye, mouth) to asymptomatic infection. Neonatal disease may be prevented by performing cesarean section on women with either active

lesions or positive viral cultures. Both HSV-1 and HSV-2 can cause severe neonatal infections that are acquired after birth from carriers handling the child. Despite their association with neonatal infections, neither HSV-1 nor HSV-2 causes congenital abnormalities to any significant degree.

Serious neonatal infection is more likely to occur when the mother is experiencing a primary herpes infection than a recurrent infection for two reasons: (1) the amount of virus produced during a primary infection is greater than during a secondary infection, and (2) mothers who have been previously infected can pass IgG across the placenta, which can protect the neonate from serious disseminated infection.

(3) Aseptic meningitis caused by HSV-2 is usually a mild, self-limited disease with few sequelae.

Both HSV-1 and HSV-2 infections are associated with erythema multiforme. The rash of erythema multiforme appears as a central red area surrounded by a ring of normal skin outside of which is a red ring (“target” or “bull’s eye” lesion). The lesions are typically macular or papular and occur symmetrically on the trunk, hands, and feet. The rash is thought to be an immune-mediated reaction to the presence of HSV antigens. Acyclovir is useful in preventing recurrent episodes of erythema multiforme, probably by reducing the amount of HSV antigens. Many drugs, especially sulfonamides among the antimicrobial drugs, commonly cause erythema multiforme. Other prominent infectious causes include *Mycoplasma pneumoniae* and viruses such as hepatitis B virus and hepatitis C virus.

Erythema multiforme major, also known as **Stevens-Johnson syndrome**, is characterized by fever, erosive oral lesions, and extensive

desquamating skin lesions. *M. pneumoniae* infection is the most common infectious cause of Stevens-Johnson syndrome.

Laboratory Diagnosis

An important diagnostic procedure is isolation of the virus from the lesion by growth in cell culture. The typical cytopathic effect occurs in 1 to 3 days, after which the virus is identified by fluorescent antibody staining of the infected cells or by detecting virus-specific glycoproteins in enzyme-linked immunosorbent assays (ELISAs). HSV-1 can be distinguished from HSV-2 by using monoclonal antibody against glycoprotein G often in an ELISA test.

A rapid presumptive diagnosis can be made from skin lesions by using the **Tzanck smear,** in which cells from the base of the vesicle are stained with Giemsa stain. The presence of multinucleated giant cells suggests herpesvirus infection .

If herpes encephalitis is suspected, a rapid diagnosis can be made by detecting HSV DNA in the spinal fluid by using a polymerase chain reaction (PCR) assay. The PCR assay is more sensitive than viral culture. The diagnosis of neonatal herpes infection typically involves the use of viral cultures or PCR assay.

Serologic tests such as the neutralization test can be used in the diagnosis of primary infections because a significant rise in antibody titer is readily observed. However, they are of no use in the diagnosis of recurrent infections because many adults already have circulating antibodies, and recurrences rarely cause a rise in antibody titer.

Treatment

Acyclovir (acycloguanosine, Zovirax) is the treatment of choice for encephalitis and systemic disease caused by HSV-1. It is also useful for

the treatment of primary and recurrent genital herpes; it **shortens the duration** of the lesions and **reduces the extent of shedding** of the virus but does *not* cure the latent state. Acyclovir is also used to treat neonatal infections caused by HSV-2. Mutants of HSV-1 resistant to acyclovir have been isolated from patients; foscarnet can be used in these cases.

For HSV-1 eye infections, other nucleoside analogues (e.g., trifluridine [Viroptic]) are used topically. Oral acyclovir is also used for HSV keratitis. Penciclovir (a derivative of acyclovir) or docosanol (a long-chain saturated alcohol) can be used to treat recurrences of orolabial HSV-1 infections in immunocompetent adults. Valacyclovir (Valtrex) and famciclovir (Famvir) are used in the treatment of genital herpes and in the suppression of recurrences.

Note that no drug treatment of the primary infection prevents recurrences; drugs have **no effect on the latent state,** but prophylactic, long-term administration of acyclovir, valacyclovir, or famciclovir can suppress clinical recurrences.

Prevention

Valacyclovir (Valtrex) and famciclovir (Famvir) are used in the suppression of recurrent lesions, especially in those with frequent recurrences caused by HSV-2. Suppressive chemoprophylaxis also reduces shedding of the virus and, as a result, transmission to others. Prevention also involves avoiding contact with the vesicular lesion or ulcer. Cesarean section is recommended for women who are at term and who have genital lesions or positive viral cultures. Circumcision reduces the risk of infection by HSV-2. There is no vaccine against HSV-1 or HSV-2.

VARICELLA-ZOSTER VIRUS (VZV)

Disease

Varicella (chickenpox) is the primary disease; zoster (shingles) is the recurrent form.

Important Properties

VZV is structurally and morphologically similar to other herpesviruses but is antigenically different. It has a single serotype. The same virus causes both varicella and zoster. Humans are the natural hosts.

Transmission & Epidemiology

The virus is transmitted by **respiratory droplets** and by direct contact with the lesions. Varicella is a highly contagious disease of childhood.

There is infectious VZV in zoster vesicles. This virus can be transmitted, usually by direct contact, to children and can cause varicella. The appearance of either varicella or zoster in a hospital is a major infection control problem because the virus can be transmitted to immunocompromised patients and cause life-threatening disseminated infection.

Pathogenesis & Immunity

VZV infects the mucosa of the upper respiratory tract, and then spreads via the blood to the skin, where the typical **vesicular rash** occurs. **Multinucleated giant cells** with intranuclear inclusions are seen in the base of the lesions. The virus infects sensory neurons and is carried by retrograde axonal flow into the cells of the **dorsal root ganglia,** where the virus becomes **latent**.

In latently infected cells, VZV DNA is located in the nucleus and is not integrated into cellular DNA. Later in life, frequently at times of reduced cell-mediated immunity or local trauma, the virus is activated and causes the vesicular skin lesions and **nerve pain** of zoster.

Immunity following varicella is lifelong: A person gets varicella only once, but zoster can occur despite this immunity to varicella. Zoster usually occurs only once. The frequency of zoster increases with advancing age, perhaps as a consequence of waning immunity.

Clinical Findings

Varicella

After an incubation period of 14 to 21 days, brief prodromal symptoms of fever and malaise occur. A papulovesicular rash then appears in crops on the trunk and spreads to the head and extremities. The rash evolves from papules to vesicles, pustules, and, finally, crusts. Itching (pruritus) is a prominent symptom, especially when vesicles are present. Varicella is mild in children but more severe in adults. Varicella pneumonia and encephalitis are the major rare complications, occurring more often in adults. **Reye's syndrome,** characterized by encephalopathy and liver degeneration, is associated with VZV and influenza B virus infection, especially in children given aspirin. Its pathogenesis is unknown.

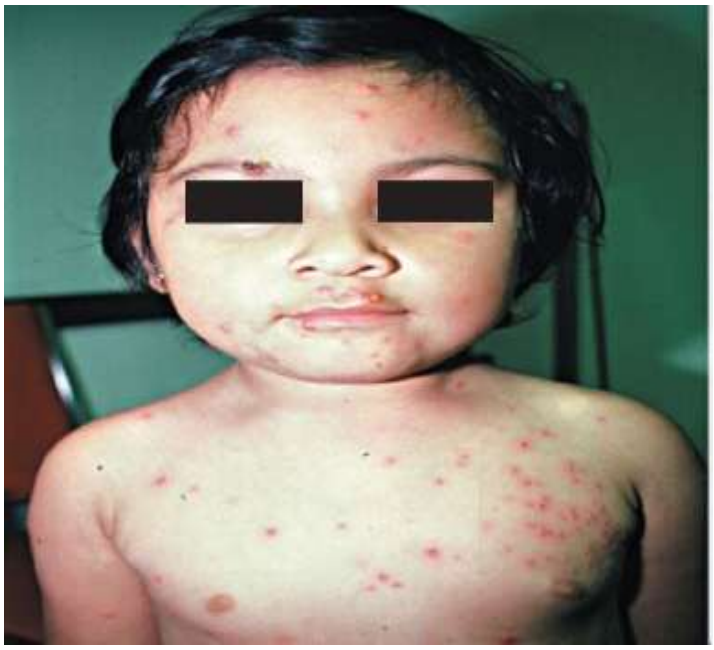


FIGURE -Varicella (chickenpox)—note vesicles on an erythematous base caused by varicella-zoster virus. (Figure courtesy of Richard P. Usatine, MD, and *The Color Atlas of Family Medicine*.)

CYTOMEGALOVIRUS (CMV)

Diseases

CMV causes cytomegalic inclusion disease (especially congenital abnormalities) in neonates. It is the **most common cause of congenital abnormalities** . CMV is a very important cause of pneumonia and other diseases in immunocompromised patients such as recipients of bone marrow and solid organ transplants.

Important Properties

CMV is structurally and morphologically similar to other herpesviruses but is antigenically different. It has a single serotype. Humans are the natural hosts; animal CMV strains do not infect humans. Giant cells are formed, hence the name *cytomegalo*.

Transmission & Epidemiology

CMV is transmitted by a **variety of modes**. Early in life, it is transmitted across the placenta, within the birth canal, and quite commonly in breast milk. In young children, its most common mode of transmission is via saliva. Later in life it is transmitted sexually; it is present in both semen and cervical secretions. It can also be transmitted during blood transfusions and organ transplants. CMV infection occurs worldwide, and more than 80% of adults have antibody against this virus.

Pathogenesis & Immunity

Infection of the fetus can cause **cytomegalic inclusion disease,** characterized by multinucleated giant cells with prominent intranuclear inclusions. Many organs are affected, and widespread congenital abnormalities result. Infection of the fetus occurs mainly when a **primary infection** occurs in the pregnant woman (i.e., when she has no antibodies that will neutralize the virus before it can infect the fetus). The fetus usually will not be infected if the pregnant woman has antibodies against the virus. Congenital abnormalities are **more common when a fetus is infected during the first trimester** than later in gestation, because the first trimester is when development of organs occurs and the death of any precursor cells can result in congenital defects.

Infections of children and adults are usually asymptomatic, except in immunocompromised individuals. CMV enters a **latent** state primarily in monocytes and can be reactivated when cell-mediated immunity is decreased. CMV can also persist in kidneys for years. Reactivation of CMV from the latent state in cervical cells can result in infection of the newborn during passage through the birth canal.

CMV has a specific mechanism of "immune evasion" that allows it to maintain the latent state for long periods. In CMV-infected cells, assembly of the major histocompatibility complex (MHC) class I–viral peptide complex is unstable, so viral antigens are not displayed on the cell surface and killing by cytotoxic T cells does not occur. In addition, CMV encodes several microRNAs, one of which binds to and prevents the translation of the cell's mRNA for the class I MHC protein. This prevents viral proteins from being displayed on the infected cell surface, and killing by cytotoxic T cells does not occur.

CMV infection causes an immunosuppressive effect by inhibiting T cells. Host defenses against CMV infection include both circulating antibody and cell-mediated immunity. Cellular immunity is more important, because its suppression can lead to systemic disease.

Clinical Findings

Approximately 20% of infants infected with CMV during gestation show clinically apparent manifestations of cytomegalic inclusion disease such as **microcephaly, seizures, deafness, jaundice, and purpura. The purpuric lesions resemble a "blueberry muffin**" and are due to **thrombocytopenia**. **Hepatosplenomegaly** is very common.

In immunocompetent adults, CMV can cause **heterophil-negative mononucleosis,** which is characterized by fever, lethargy, and the presence of abnormal lymphocytes in peripheral blood smears. Systemic CMV infections, especially pneumonitis, esophagitis, and hepatitis, occur in a high proportion of immunosuppressed individuals (e.g., those with renal and bone marrow transplants). In patients with acquired immunodeficiency syndrome (AIDS), CMV commonly infects the intestinal tract and causes intractable colitis with diarrhea. CMV also causes retinitis in AIDS patients, which can lead to blindness.

Laboratory Diagnosis

The preferred approach involves culturing in special tubes called **shell vials** coupled with the use of immunofluorescent antibody, which can make a diagnosis in 72 hours. The virus obtained in the culture can then be used to determine the drug susceptibility to ganciclovir.

Other diagnostic methods include fluorescent antibody and histologic staining of inclusion bodies in giant cells in urine and in tissue. The inclusion bodies are intranuclear and have an oval **owl**'**s eye** shape. A fourfold or greater rise in antibody titer is also diagnostic. PCR-based assays for CMV DNA or RNA in tissue or body fluids, such as spinal fluid and amniotic fluid, are also very useful.

Treatment

Ganciclovir (Cytovene) is moderately effective in the treatment of CMV retinitis. Valganciclovir, which can be taken orally, is also effective against CMV retinitis. CMV strains resistant to ganciclovir and valganciclovir have emerged.

Fomivirsen (Vitravene) is an antisense DNA approved for the intraocular treatment of CMV retinitis. It is the first and, at present, the only antisense molecule to be approved for the treatment of human disease.

EPSTEIN–BARR VIRUS (EBV)

Diseases

EBV causes infectious mononucleosis. It is associated with Burkitt's lymphoma, other B-cell lymphomas, and nasopharyngeal carcinoma. EBV also causes hairy leukoplakia.

Important Properties

EBV is structurally and morphologically similar to other herpesviruses but is antigenically different. The most important antigen is the **viral capsid antigen** (VCA), because it is used most often in diagnostic tests. The early antigens (EA), which are produced prior to viral DNA

synthesis, and Epstein–Barr nuclear antigen (EBNA), which is located in the nucleus bound to chromosomes, are sometimes diagnostically helpful as well. Two other antigens, lymphocyte-determined membrane antigen and viral membrane antigen, have been detected also. Neutralizing activity is directed against the viral membrane antigen.

Humans are the natural hosts. EBV infects mainly lymphoid cells, primarily **B lymphocytes**. EBV also infects the epithelial cells of the pharynx, resulting in the prominent sore throat. In latently infected cells, EBV DNA is in the nucleus and is not integrated into cellular DNA. Some, but not all, genes are transcribed, and only a subset of those are translated into protein.

Transmission & Epidemiology

EBV is transmitted primarily by the **saliva** . The saliva of people with a reactivation of a latent infection as well as people with an active infection can serve as a source of the virus. In contrast to CMV, blood transmission of EBV is very rare.

Pathogenesis & Immunity

The infection first occurs in the oropharynx and then spreads to the blood, where it **infects B lymphocytes**. Cytotoxic T lymphocytes react against the infected B cells. The T cells are the “atypical lymphs” seen in the blood smear. EBV remains **latent within B lymphocytes**.

The immune response to EBV infection consists first of IgM antibody to the VCA. IgG antibody to the VCA follows and persists for life. The IgM response is therefore useful for diagnosing acute infection, whereas the IgG response is best for revealing prior infection. Lifetime immunity

against second episodes of infectious mononucleosis is based on antibody to the viral membrane antigen.

In addition to the EBV-specific antibodies, nonspecific **heterophil antibodies** are found. The term *heterophil* refers to antibodies that are detected by tests using antigens different from the antigens that induced them. The heterophil antibodies formed in infectious mononucleosis agglutinate sheep or horse red blood cells in the laboratory. (Cross-reacting Forssman antibodies in human serum are removed by adsorption with guinea pig kidney extract prior to agglutination.) Note that these antibodies do not react with any component of EBV. It seems likely that EBV infection modifies a cell membrane constituent such that it becomes antigenic and induces the heterophil antibody. Heterophil antibodies usually disappear within 6 months after recovery. These antibodies are not specific for EBV infection and are also seen in individuals with hepatitis B and serum sickness.

Clinical Findings

Infectious mononucleosis is characterized primarily by fever, sore throat, lymphadenopathy, and splenomegaly. Anorexia and lethargy are prominent. Hepatitis is frequent; encephalitis occurs in some patients. Spontaneous recovery usually occurs in 2 to 3 weeks. Splenic rupture, associated with contact sports such as football, is a feared but rare complication of the splenomegaly.

In addition to the common form of infectious mononucleosis described in the previous paragraph, EBV causes a severe, often fatal, progressive form of infectious mononucleosis that occurs in children with an inherited immunodeficiency called X-linked lymphoproliferative syndrome. The

mutated gene encodes a signal transduction protein required for both T-cell and natural killer–cell function. The mortality rate is 75% by age 10. Bone marrow or cord blood transplants may cure the underlying immunodeficiency. EBV also causes **hairy leukoplakia**—a whitish, nonmalignant lesion with an irregular "hairy" surface on the lateral side of the tongue. It occurs in immunocompromised individuals, especially AIDS patients.

EBV infection is associated with several cancers, namely Burkitt's lymphoma and nasopharyngeal carcinoma.

Another EBV-associated disease is post-transplant lymphoproliferative disorder (PTLD). The most common form of PTLD is a B-cell lymphoma. PTLD occurs following both bone marrow transplants and solid organ transplants. The main predisposing factor to PTLD is the immunosuppression required to prevent rejection of the graft. The lymphoma will regress if the degree of immunosuppression is reduced.

Laboratory Diagnosis

The diagnosis of infectious mononucleosis in the clinical laboratory is based primarily on two approaches:

(1) In the **hematologic** approach, absolute lymphocytosis occurs, and as many as 30% abnormal lymphocytes are seen on a smear. These **atypical lymphs** are enlarged, have an expanded nucleus, and an abundant, often vacuolated cytoplasm. They are cytotoxic T cells that are reacting against the EBV-infected B cells.

(2) In the **immunologic** approach, there are two types of serologic tests: (a) The **heterophil antibody** test is useful for the early diagnosis of

infectious mononucleosis because it is usually positive by week 2 of illness. However, because the antibody titer declines after recovery, it is not useful for detection of prior infection. The Monospot test is often used to detect the heterophil antibody; it is more sensitive, more specific, and less expensive than the tube agglutination test. (b) The **EBV-specific antibody tests** are used primarily in diagnostically difficult cases. The IgM VCA antibody response can be used to detect early illness; the IgG VCA antibody response can be used to detect prior infection. In certain instances, antibodies to EA and EBNA can be useful diagnostically.

Although EBV can be isolated from clinical samples such as saliva by morphologic transformation of cord blood lymphocytes, it is a technically difficult procedure and is not readily available. No virus is synthesized in the cord lymphocytes; its presence is detected by fluorescent antibody staining of the nuclear antigen.

Treatment

No antiviral therapy is necessary for uncomplicated infectious mononucleosis. Acyclovir has little activity against EBV, but administration of high doses may be useful in life-threatening EBV infections.

Association With Cancer

EBV infection is associated with cancers of lymphoid origin: **Burkitt's lymphoma** in African children, other B-cell lymphomas, nasopharyngeal carcinoma .

Human Herpes 8 (Kaposi's Sarcoma – Assosiated Herpes Virus):-

In 1994, it was reported that a new herpesvirus, now known as human herpesvirus 8 (HHV-8), or Kaposi's sarcoma–associated herpesvirus (KSHV), causes Kaposi's sarcoma (KS), the most common cancer in patients with AIDS. The idea that a virus other than HIV is the cause of KS arose from epidemiologic data showing that KS was common in patients who acquired HIV sexually but rare in patients who acquired HIV via blood transfusion. A second virus transmitted sexually appeared likely to be the cause.

The initial evidence that HHV-8 was involved was the finding that most KS cells taken from AIDS patients contain the DNA of this virus, but tissues taken from AIDS patients without KS had very little viral DNA. The DNA of this virus was also found in KS cells that arose in non–HIV-infected patients. HHV-8 causes malignant transformation by a mechanism similar to that of other DNA viruses (e.g., human papillomavirus), namely, inactivation of a tumor suppressor gene. A protein encoded by HHV-8 called latency-associated nuclear antigen (LANA) inactivates RB and p53 tumor suppressor proteins, which causes malignant transformation of endothelial cells.

Transmission of HHV-8 occurs primarily via sex and by saliva, but it is also transmitted in transplanted organs such as kidneys and appears to be the cause of transplantation-associated KS. The DNA of HHV-8 is found in the cells of transplantation-associated KS but not in the cells of other transplantation-associated cancers.

KS in AIDS patients is a malignancy of vascular endothelial cells that contains many spindle-shaped cells and erythrocytes. The lesions are reddish to dark purple, flat to nodular, and often appear at multiple sites such as the skin, oral cavity, and soles (but not the palms), lesions occur

commonly in the gastrointestinal tract and the lungs. The extravasated red cells give the lesions their purplish color. HHV-8 also infects B cells, inducing them to proliferate and produce a type of lymphoma called primary effusion lymphoma.

Laboratory diagnosis of KS is often made by biopsy of the skin lesions. HHV-8 DNA and RNA are present in most spindle cells, but that analysis is not usually done. Virus is not grown in culture.

The type of treatment depends on the site and number of the lesions. Surgical excision, radiation, and systemic drugs, such as alpha interferon or vinblastine, can be used. There is no specific antiviral therapy and no vaccine against HHV-8.