Virology

Post-Graduate Course
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In This Lecture:

- Enveloped DNA Viruses
  - Herpesviruses

Introduction

- The herpesvirus family contains several of the most important human viral pathogens.
- Clinically, the herpesviruses exhibit a spectrum of diseases.
- Some have a wide host-cell range, whereas others have a narrow host-cell range.
- Ability to establish lifelong persistent infections in their hosts and to undergo periodic reactivation. Their frequent reactivation in immunosuppressed patients causes serious health complications.
- Herpesviruses possess a large number of genes, some of which have proved to be susceptible to antiviral chemotherapy.

Properties of Herpesviruses

Important properties of herpesviruses are summarized in Table 33

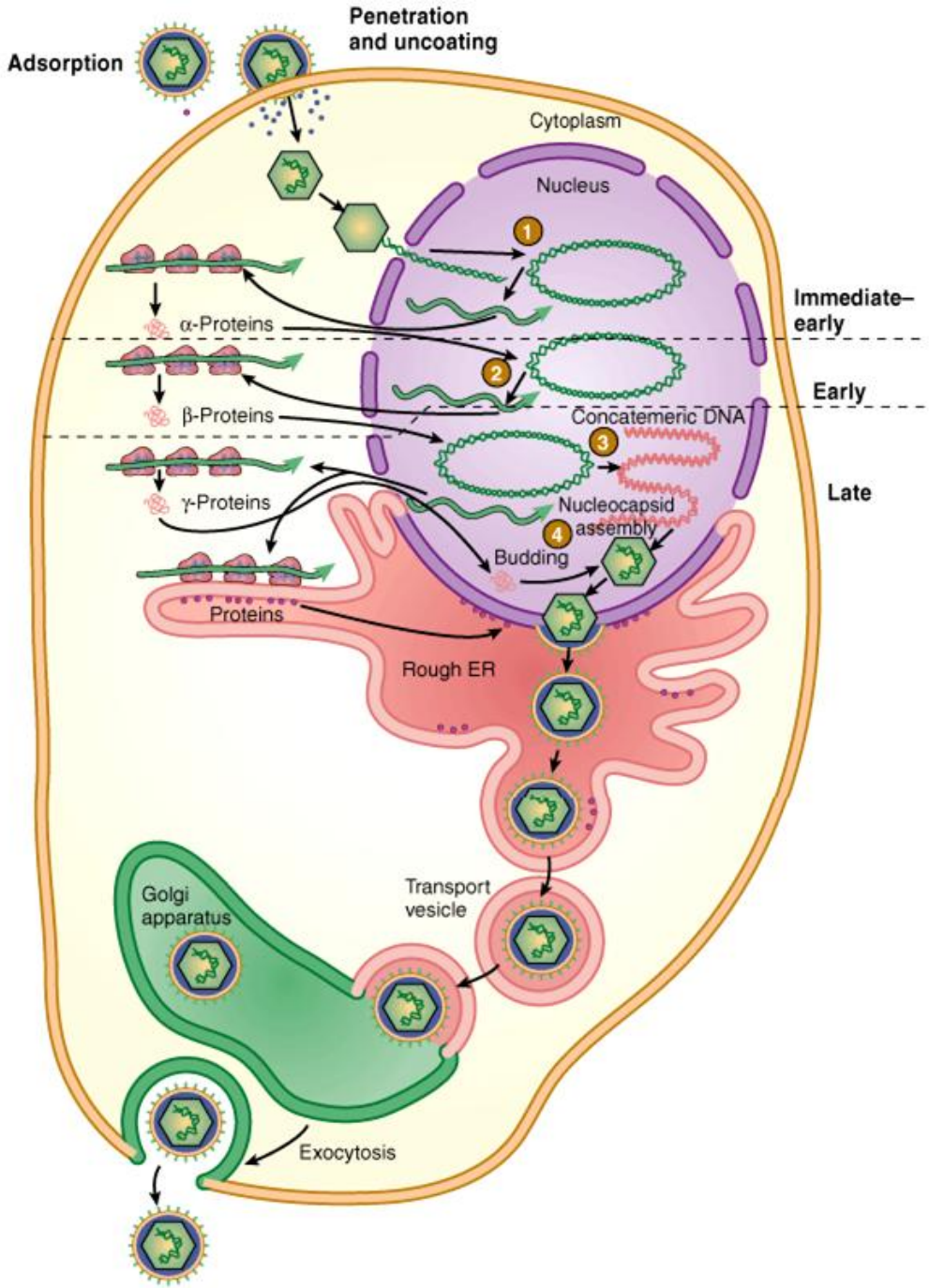
Table 33 Important Properties of Herpesviruses
Virion: Spherical, 150â€“200 nm in diameter (icosahedral)
Genome: Double-stranded DNA, linear
Proteins: More than 35 proteins in virion
Envelope: Contains viral glycoproteins, Fc receptors
Replication: Nucleus, bud from nuclear membrane
Outstanding characteristics:
Encode many enzymes
Establish latent infections
Persist indefinitely in infected hosts
Frequently reactivated in immunosuppressed hosts
Some are cancer-causing

Structure & Composition

- All herpesviruses have a core of double-stranded DNA.
- protein coat that exhibits icosahedral symmetry and has 162 capsomeres.
- The nucleocapsid is surrounded by an envelope that is derived from the nuclear membrane of the infected cell and contains viral glycoprotein spikes

Classification

Classification of Human Herpesviruses					
	Biologic Properties			**Examples**	
Subfamily ("-herpesvirinae')	**Growth Cycle and Cytopathology**	**Latent Infections**	**Genus ("-*virus*")**	**Official Name ("Human - Herpesvirus")**	**Common Name**
Alpha	Short, cytolytic	Neurons	*Simplex*	1	Herpes simplex virus type 1
				2	Herpes simplex virus type 2
			Varicello	3	Varicella-zoster virus
Beta	Long, cytomegalic	Glands, kidneys	*Cytomegalo*	5	Cytomegalovirus
	Long, lymphoproliferative	Lymphoid tissue	*Roseolo*	6	Human herpesvirus 6
				7	Human herpesvirus 7
Gamma	Variable, lymphoproliferative	Lymphoid tissue	*Lymphocrypto*	4	Epstein-Barr virus
			Rhadino	8	Kaposi sarcoma-associated herpesvirus


Adsorption
Penetration and uncoating
Cytoplasm
Nucleus
1
α-Proteins
Immediate–early
2
Early
β-Proteins
Concatemeric DNA
3
Late
γ-Proteins
Nucleocapsid assembly
4
Budding
Proteins
Rough ER
Golgi apparatus
Transport vesicle
Exocytosis

Herpesvirus Diseases

- **HSV-1 and HSV-2** infect epithelial cells and establish latent infections in neurons. Type 1 is classically associated with oropharyngeal lesions and causes recurrent attacks of "fever blisters." Type 2 primarily infects the genital mucosa and is mainly responsible for genital herpes. Both viruses also cause neurologic disease. HSV-1 is the leading cause of sporadic encephalitis in the United States. Both type 1 and type 2 can cause neonatal infections which are often severe.
- **Varicella-zoster virus** causes chickenpox (varicella) on primary infection and establishes latent infection in neurons. Upon reactivation, the virus causes zoster (shingles). Adults who are infected for the first time with varicella-zoster virus are apt to develop serious viral pneumonia.

- **Cytomegalovirus** replicates in epithelial cells of the respiratory tract, salivary glands, and kidneys and persists in lymphocytes. It causes an infectious mononucleosis. In newborns, cytomegalic inclusion disease may occur. It is an important cause of congenital defects and mental retardation**.**
- **Human herpesvirus 6** infects T lymphocytes. It is typically acquired in early infancy and causes exanthem subitum (roseola infantum). **Human herpesvirus 7**, also a T-lymphotropic virus, has not yet been linked to any specific disease**.**
- **EBV** replicates in epithelial cells of the oropharynx and parotid gland and establishes latent infections in lymphocytes. It causes infectious mononucleosis and is the cause of human lymphoproliferative disorders, especially in immunocompromised patients.
- **Human herpesvirus 8** appears to be associated with the development of Kaposi sarcoma, a vascular tumor that is common in patients with AIDS**..**

Herpesvirus Diseases

- Human herpesviruses are frequently reactivated in immunosuppressed patients (eg, transplant recipients, cancer patients) and may cause severe disease, such as pneumonia or lymphomas.

- Herpesviruses have been linked with malignant diseases in humans and lower animals: EBV with Burkitt lymphoma of African children, with nasopharyngeal carcinoma, and with other lymphomas; KSHV with Kaposi sarcoma.

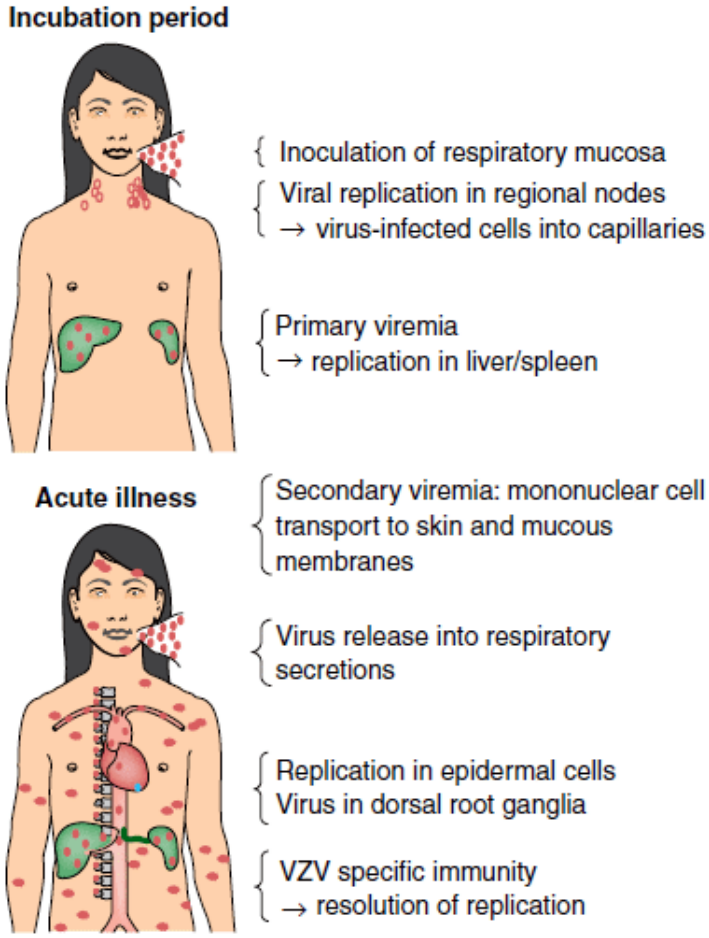
Varicella-Zoster Virus

- **Varicella (chickenpox)** is a mild, highly contagious disease, chiefly of children, characterized clinically by a generalized vesicular eruption of the skin and mucous membranes. The disease may be severe in adults and in immunocompromised children**.**

- **Zoster (shingles)** is a sporadic, incapacitating disease of adults or immunocompromised individuals that is characterized by a rash limited in distribution to the skin innervated by a single sensory ganglion. The lesions are similar to those of varicella**.**

- 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 partially immune host to reactivation of varicella virus present in latent form in neurons in sensory ganglia**.**

Varicella-Zoster Virus



The pathogenesis of primary infection with varicella-zoster virus (VZV). The incubation period with primary viremia lasts from 10 to 21 days. A secondary viremic phase results in the transport of virus to skin and respiratory mucosal sites. Replication in epidermal cells causes the characteristic rash of varicella, referred to as chickenpox. The induction of varicella-zoster virus-specific immunity is required to terminate viral replication. The virus gains access to cells of the trigeminal and dorsal root ganglia during primary infection and establishes latency. (Reproduced with permission from Arvin AM: Varicella-zoster virus. In: *Fields Virology*, 3rd ed. Fields BN et al [editors]. Lippincott-Raven, 1996.)

Immunity

- Varicella and zoster viruses are identical, the two diseases being the result of differing host responses. Previous infection with varicella is believed to confer lifelong immunity to varicella. Antibodies induced by varicella vaccine persist for at least 20 years. Zoster occurs in the presence of neutralizing antibody to varicella.
- Increases in varicella antibody titer may occur in persons with HSV infections.
- The development of varicella-zoster virus-specific cell-mediated immunity is important in recovery from both varicella and zoster. Appearance of local interferon may also contribute to recovery.
- Varicella-zoster virus, like other herpesviruses, encodes means of evading host immune responses. For example, it downregulates major histocompatibility complex class I and II antigen expression.

Epidemiology

- Varicella and zoster occur worldwide. Varicella (chickenpox) is highly communicable and is a common epidemic disease of childhood (most cases occur in children under 10 years of age).
- Adult cases do occur. It is much more common in winter and spring than in summer in temperate climates.
- Zoster occurs sporadically, chiefly in adults and without seasonal prevalence. Ten to 20 percent of adults will experience at least one zoster attack during their lifetime, usually after the age of 50.

- A live attenuated varicella vaccine is available. Since the vaccine was introduced in 1995, there has been a steady decline in the incidence of varicella diseases; however, varicella outbreaks continue to occur among school children, because some children are unvaccinated and the vaccine is 80-85% effective in vaccinated persons.

- Varicella spreads readily by airborne droplets and by direct contact. A varicella patient is probably infectious (capable of transmitting the disease) from shortly before the appearance of rash to the first few days of rash. Contact infection is less common in zoster, perhaps because the virus is absent from the upper respiratory tract in typical cases. Zoster patients can be the source of varicella in susceptible children. Varicella-zoster virus DNA has been detected, using a PCR amplification method, in air samples from hospital rooms of patients with active varicella (82%) and zoster (70%) infections.

Cytomegalovirus

- Cytomegaloviruses are the agents of the most common congenital infection .
- Cytomegalic inclusion disease is a generalized infection of infants caused by intrauterine or early postnatal infection with the cytomegaloviruses.
- The name for the classic cytomegalic inclusion disease derives from the propensity for massive enlargement of cytomegalovirus-infected cells. Cytomegalovirus poses an important public health problem because of its high frequency of congenital infections, which may lead to severe congenital anomalies.
- Inapparent infection is common during childhood and adolescence. Severe cytomegalovirus infections are frequently found in adults who are immunosuppressed.

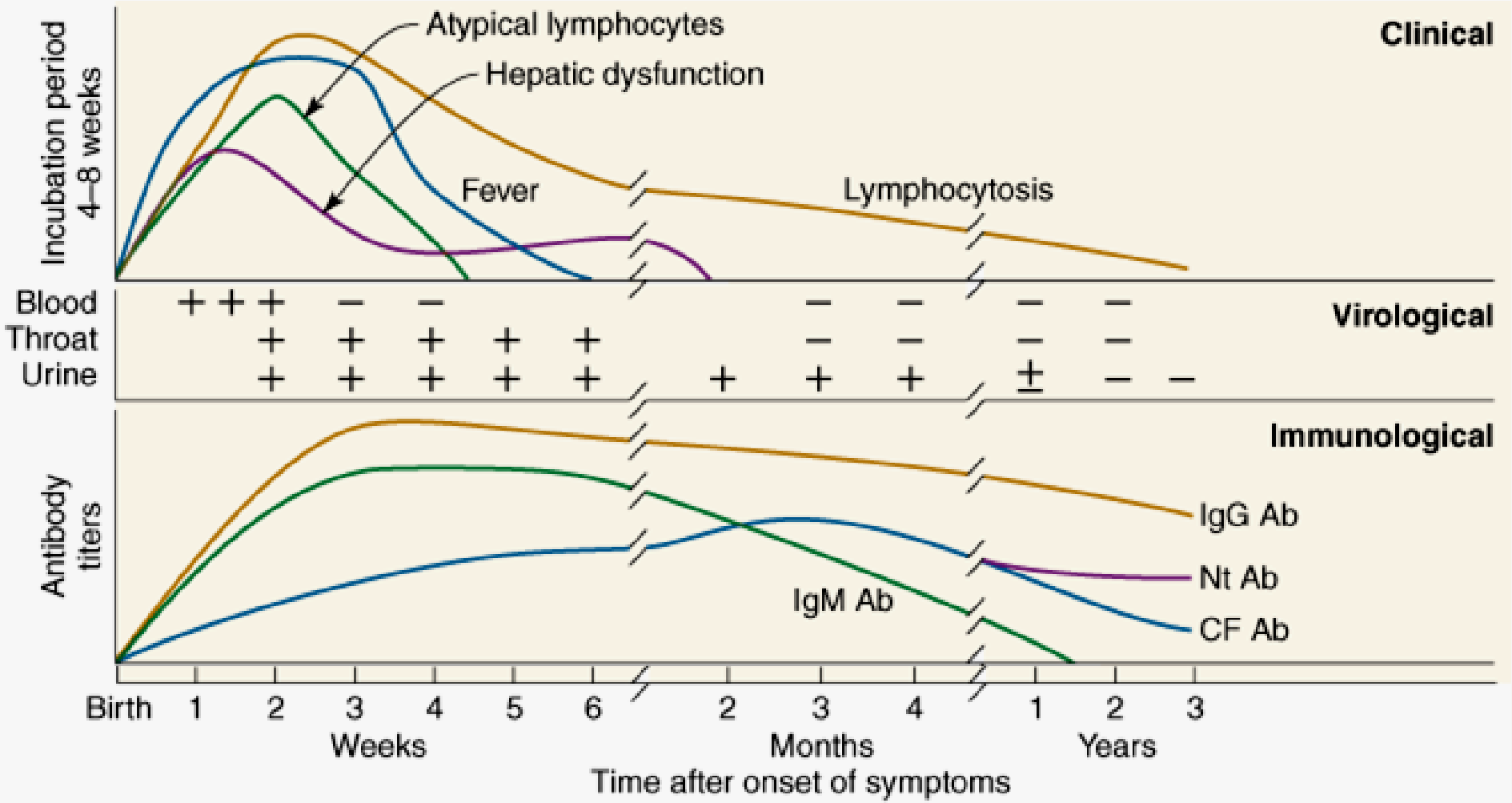
Properties of the Virus

- **Cytomegalovirus** has the largest genetic content of the human herpesviruses. Only a few of the many proteins encoded by the virus (over 200) have been characterized. One, a cell surface glycoprotein, acts as an **Fc receptor** that can nonspecifically bind the Fc portion of immunoglobulins. This may help infected cells evade immune elimination by providing a protective coating of irrelevant host immunoglobulins**.**
- The major immediate-early promoter-enhancer of cytomegalovirus is one of the strongest known **enhancers**, due to the concentration of binding sites for cellular transcription factors. It is used experimentally to support high-level expression of foreign genes**.**
- Cytomegaloviruses are very **species-specific and cell type-specific**. All attempts to infect animals with human cytomegalovirus have failed. A number of animal cytomegaloviruses exist, all of them species-specific**.**

Pathogenesis & Pathology

- Cytomegalovirus may be **transmitted** person-to-person in several different ways, all requiring close contact with virus-bearing material.
- There is a 4- to 8-week **incubation period** in normal older children and adults after viral exposure.
- The virus causes a **systemic infection**; it has been isolated from lung, liver, esophagus, colon, kidneys, monocytes, and T and B lymphocytes.
- The disease is an **infectious mononucleosis-like syndrome**, although most cytomegalovirus infections are subclinical.
- 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.

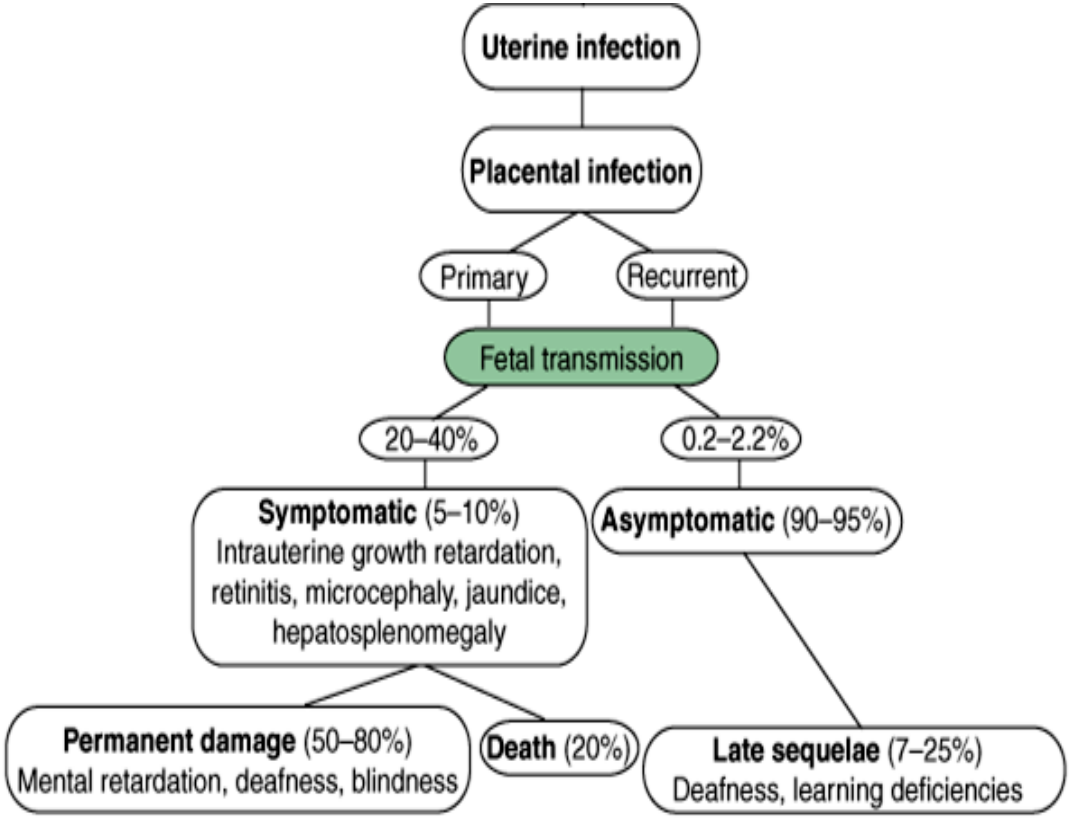
Cytomegalovirus



Immunosuppressed Hosts

- Primary cytomegalovirus infections in immunosuppressed hosts are much more severe than in normal hosts. Individuals at greatest risk for cytomegalovirus disease are those receiving organ transplants, those with malignant tumors who are receiving chemotherapy, and those with AIDS. Viral excretion is increased and prolonged, and the infection is more apt to become disseminated. Pneumonia is the most common complication.
- The host immune response presumably maintains cytomegalovirus in a latent state in seropositive individuals. Reactivated infections are associated with disease much more often in immunocompromised patients than in normal hosts. Although usually less severe, reactivated infections may be as virulent as primary infections.

Congenital & Perinatal Infections



Source: Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA: *Jawetz, Melnick, & Adelberg's Medical Microbiology, 25th Edition*: http://www.accessmedicine.com

Congenital infections by cytomegalovirus and birth defects in symptomatic and asymptomatic children. Cytomegalovirus is the most common intrauterine infection associated with congenital defects. (Reproduced with permission from Pereira L et al: Insights into viral transmission at the uterine-placental interface. Trends Microbiol 2005;13:164.)

Immunocompromised Hosts: Clinically

- Both morbidity and mortality rates are increased with primary and recurrent cytomegalovirus infections in immunocompromised individuals.
- Pneumonia is a frequent complication. It occurs in 10-20% of bone marrow transplant recipients.
- Virus-associated leukopenia is common in solid organ transplant recipients; also seen are obliterative bronchiolitis in lung transplants, graft atherosclerosis after heart transplantation, and cytomegalovirus-related rejection of renal allografts.
- Cytomegalovirus often causes disseminated disease in untreated AIDS patients; gastroenteritis and chorioretinitis are common problems, the latter often leading to progressive blindness.

Epidemiology

- Cytomegalovirus is endemic in all parts of the world; epidemics are unknown. It is present throughout the year, with no seasonal variation seen in infection rates.
- The prevalence of infection varies with socioeconomic status, living conditions, and hygienic practices. Antibody prevalence may be moderate (40-70%) in adults in high socioeconomic groups in developed countries, in contrast to a prevalence of 90% in children and adults in developing nations and in low socioeconomic groups in developed countries.
- New infections are almost always asymptomatic. After infection, virus is shed from multiple sites. Viral shedding may continue for years, often intermittently, as latent virus becomes reactivated.

Treatment & Control

- Drug treatments of cytomegalovirus infections have shown some encouraging results. Ganciclovir, a nucleoside structurally related to acyclovir, has been used successfully to treat life-threatening cytomegalovirus infections in immunosuppressed patients .
- Both live and recombinant cytomegalovirus vaccines are under development.

Epstein-Barr Virus

- EBV 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.

Biology of Epstein-Barr Virus

- The major target cell for EBV is the **B lymphocyte**. When human B lymphocytes are infected with EBV, continuous cell lines can be established, indicating that cells have been **immortalized** by the virus. Very few of the immortalized cells produce infectious virus.

- EBV initiates infection of B cells by **binding** to the viral receptor, which is the receptor for the C3d component of complement (CR2 or **CD21**). EBV directly enters a **latent** state in the lymphocyte without undergoing a period of complete viral replication. The hallmarks of **latency** are viral persistence, restricted virus expression, and the potential for reactivation and lytic replication.

Biology of Epstein-Barr Virus

- When virus binds to the cell surface, cells are **activated** to enter the cell cycle. Subsequently, a limited repertoire of EBV genes are expressed, and the cells are able to proliferate indefinitely.
- The linear EBV genome forms a **circle** and is amplified during the **cell cycle S phase**; the majority of viral DNA in the immortalized cells exists as circular form.

Biology of Epstein-Barr Virus

- EBV-immortalized B lymphocytes express differentiated functions, such as secretion of **immunoglobulin**.
- B cell activation products (eg, **CD23**) are also expressed.
- Several patterns of **latent viral gene expression** are recognized, based on the spectrum of proteins and transcripts expressed. These include:
  - EBV nuclear antigens (EBNA1, 2, 3A-3C, LP),
  - latent membrane proteins (LMP1, 2), and
  - small untranslated RNAs (EBERs).

Viral Antigens

EBV **antigens** are divided into three classes:

- (1) **Latent phase antigens** are synthesized by latently infected cells. These include the EBNAs and the LMPs. Their expression reveals that an EBV genome is present. Only EBNA1, needed to maintain the viral DNA episomes, is invariably expressed; expression of the other latent phase antigens may be regulated in different cells. LMP1 mimics an activated growth factor receptor.
- (2) **Early antigens** are nonstructural proteins whose synthesis is not dependent on viral DNA replication. The expression of early antigens indicates the onset of productive viral replication.
- (3) **Late antigens** are the structural components of the viral capsid (viral capsid antigen) and viral envelope (glycoproteins). They are produced abundantly in cells undergoing productive viral infection**.**

Pathogenesis & Pathology

- **Primary Infection**

EBV is commonly transmitted by infected saliva and initiates infection in the oropharynx. Viral replication occurs in epithelial cells (or surface B lymphocytes) of the pharynx and salivary glands. Many people shed low levels of virus for weeks to months after infection. Infected B cells 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

Primary infections in children are usually subclinical, but if they occur in young adults acute infectious mononucleosis often develops. Mononucleosis is a polyclonal stimulation of lymphocytes. EBV-infected B cells synthesize immunoglobulin.

Pathogenesis & Pathology

- **Reactivation from Latency**

Reactivations of EBV latent infections can occur, as evidenced by increased levels of virus in saliva and of DNA in blood cells. These are usually clinically silent. Immunosuppression is known to reactivate infection, sometimes with serious consequences.

Clinical Findings

- **Infectious Mononucleosis**

The typical illness is self-limited and lasts 2-4 weeks. During the disease, there is an increase in the number of circulating white blood cells, with a predominance of lymphocytes. Many of these are large, **atypical T lymphocytes**. Low-grade fever and malaise may persist for weeks to months after acute illness.

Clinical Findings: ***Cancer***

- Sera from patients with Burkitt lymphoma or nasopharyngeal carcinoma contain elevated levels of antibody to virus-specific antigens, and the tumor tissues contain EBV DNA and express a limited number of viral genes.
- Burkitt lymphoma is a tumor of the jaw in African children and young adults. Most African tumors (>90%) contain EBV DNA and express EBNA1 antigen. In other parts of the world, only about 20% of Burkitt lymphomas contain EBV DNA. It is speculated that EBV may be involved at an early stage in Burkitt lymphoma by immortalizing B cells.
- Finally, there are characteristic chromosome translocations that involve immunoglobulin genes and result in deregulation of expression of the c-*myc* proto-oncogene

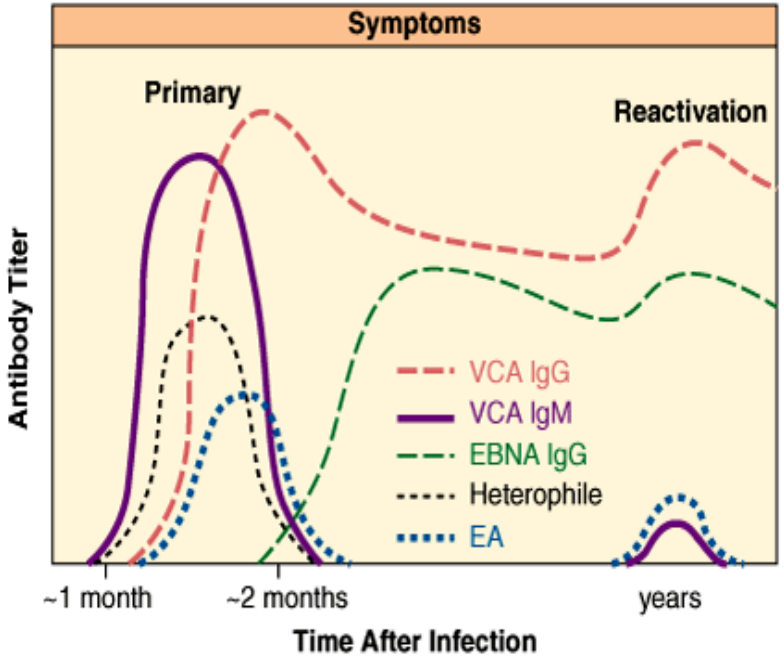
Clinical Findings: ***Cancer***

- Nasopharyngeal carcinoma is a cancer of epithelial cells and is common in males of Chinese origin.
- EBV DNA is regularly found in nasopharyngeal carcinoma cells, and patients have high levels of antibody to EBV.
- EBNA1 and LMP1 are expressed.
- Genetic and environmental factors are believed to be important in the development of nasopharyngeal carcinoma.

Clinical Findings: ***Cancer***

- Immunodeficient patients are susceptible to EBV-induced lymphoproliferative diseases that may be fatal. From 1% to 10% of transplant patients develop an EBV-associated lymphoproliferative disorder, often when experiencing a primary infection. Aggressive monoclonal B cell lymphomas may develop.
- AIDS patients are susceptible to EBV-associated lymphomas and oral hairy leukoplakia, a wart-like growth that develops on the tongue; it is an epithelial focus of EBV replication. Virtually all central nervous system non-Hodgkin lymphomas are associated with EBV, whereas less than 50% of systemic lymphomas are EBV-positive.
- In addition, EBV is associated with classic Hodgkin disease, with the viral genome detected in the malignant Reed-Sternberg cells in up to 50% of cases.

Epstein-Barr Virus



Source: Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA: *Jawetz, Melnick, & Adelberg's Medical Microbiology, 25th Edition*: http://www.accessmedicine.com

Typical pattern of antibody formation to EBV-specific antigens after a primary infection. Individuals with recent infection have IgM and IgG antibodies to the viral capsid antigen (VCA IgM, VCA IgG); only the IgG antibodies persist for years. Transient heterophil antibodies develop that can agglutinate sheep cells. Antibodies to early antigens (EA) develop in many patients and persist for several months. Several weeks after acute infection, antibodies to EBV nuclear-associated antigens (EBNA) and membrane antigen appear and persist for life. (Reprinted from Gulley ML, Tang W: Laboratory assays for Epstein-Barr virus-related disease. J Mol Diagnost 2008;10:279â€“292 with permission from the American Society for Investigative Pathology and the Association for Molecular Pathology.)

Epidemiology

- EBV is common in all parts of the world, with over 90% of adults being seropositive.
- It is transmitted primarily by contact with oropharyngeal secretions.
- In developing areas, infections occur early in life; more than 90% of children are infected by age 6. These infections in early childhood usually occur without any recognizable disease. The inapparent infections result in permanent immunity to infectious mononucleosis.
- In industrialized nations, more than 50% of EBV infections are delayed until late adolescence and young adulthood. In almost half of cases, the infection is manifested by infectious mononucleosis.

Human Herpesvirus 6

- The T-lymphotropic human herpesvirus 6 was first recognized in 1986 .
- Isolates of human herpesvirus 6 segregate into two closely related but distinct antigenic groups (designated A and B.(
- The virus grows well in CD_4 T lymphocytes. Other cell types also support viral replication, including B cells and cells of glial, fibroblastoid, and megakaryocyte origin. Cells in the oropharynx must become infected, since virus is present in saliva. It is not known which cells in the body become latently infected.
- Human CD_{46} is the cellular receptor for the virus.

Epidemiology & Clinical Findings

- It is estimated that over 90% of children over age 1 and adults are virus positive.
- Infections with human herpesvirus 6 typically occur in early childhood. This primary infection causes exanthem subitum (roseola infantum, or "sixth disease"), the mild common childhood disease characterized by high fever and skin rash. The 6B variant appears to be the cause of this disease. The virus is associated with febrile seizures in children.

Human Herpesvirus 7

- A T-lymphotropic human herpesvirus, designated human herpesvirus 7, was first isolated in 1990 from activated T cells recovered from peripheral blood lymphocytes of a healthy individual.
- Human herpesvirus 7 is immunologically distinct from human herpesvirus 6, though they share about 50% homology at the DNA level.
- Human herpesvirus 7 appears to be a ubiquitous agent, with most infections occurring in childhood but later than the very early age of infection noted with human herpesvirus 6.
- Persistent infections are established in salivary glands, and the virus can be isolated from saliva of most individuals.
- Similar to human herpesvirus 6, primary infection with human herpesvirus 7 has been linked with roseola infantum in infants and young children.
- Any other disease associations of human herpesvirus 7 remain to be established.

Human Herpesvirus 8

- A new herpesvirus, human herpesvirus 8 and also called KSHV, was first detected in 1994 in Kaposi sarcoma specimens. KSHV is lymphotropic and is more closely related to EBV than to other known herpesviruses.
- The KSHV genome contains numerous genes related to cellular regulatory genes involved in cell proliferation, apoptosis, and host responses (cyclin D, cytokines, chemokine receptor) that presumably contribute to viral pathogenesis. KSHV is the cause of Kaposi sarcomas, vascular tumors of mixed cellular composition, and is involved in the pathogenesis of body cavity-based lymphomas occurring in AIDS patients.
- Contact with oral secretions is likely the most common route of transmission. The virus can also be transmitted sexually, vertically, by blood, and through organ transplants. Viral DNA has also been detected in breast-milk samples in Africa. Infections are common in Africa (>50%) and are acquired early in life.

Human Herpesvirus 8

- Viral DNA can be detected in patient specimens using PCR assays. Direct virus culture is difficult and impractical. Serologic assays are available to measure persistent antibody to KSHV, using indirect immunofluorescence, Western blot, and ELISA formats.
- Foscarnet, ganciclovir, and cidofovir have activity against KSHV replication. The rate of new Kaposi sarcomas is markedly reduced in HIV-positive patients on effective antiretroviral therapy, probably reflecting reconstituted immune surveillance against KSHV-infected cells.