**XIV Lecture: Human pathogen viruses as Herpesviruses, Picornaviruses and Rhabdoviruses. Arboviruses.**

**The purpose of the lecture.** To provide information on the morpho-biological characteristics of herpesviruses, picornaviruses, rhabdoviruses and arboviruses, diseases which caused this viruses , microbiological diagnosis, treatment and prevention.

**Lecture plan:**

1. Herpesviruses. Classification. Structure and cultivation of virion. Simple herpes viruses. Chickenpox virus and herpes zoster. Cytomegalovirus. Epstein-Barr virus. Morpho-biological features, diseases. Microbiological diagnosis.

2. Picornaviruses. General features, classification.

- Enteroviruses. Polio virus and caused diseases.Specific prevention and treatment. Coxsackie and ECHO viruses.

3. Rhabdoviruses. The rabies virus. Virion structure. Microbiological diagnosis of rabies, specific prevention.

4. Arboviruses. General features.

- Togaviruses, Flaviviruses, Bunyaviruses, Filoviruses. General features. Classification. The role in human pathology. Microbiological diagnosis, specific prevention and treatment.

Lecture equipment: Computer, projector, electronic slides

Literature: p.1

**HERPESVIRUSES**

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). 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 are large (120-200 nm in diameter), second in size only to poxviruses. They replicate in the nucleus, form intranuclear inclu-sions, 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 nucleo-capsid 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.

Three of the herpesviruses, HSV types 1 and 2 and var-icella-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 .

*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.

***Important Properties***

HSV-1 and HSV-2 are structurally and morphologically indistinguishable. They can, however, be differentiated by the restriction endonuclease patterns of their genome DNA and by type-specific monoclonal antisera against glycoprotein G. Humans are the natural hosts of both HSV-1 and HSV-2.

*Summary of Replicative Cycle*

The cycle begins when HSV-1 binds first to heparan sulfate on the cell surface and then to a second receptor, nectin. Following fusion of the viral envelope with the cell mem-brane, the nucleocapsid and the tegument proteins are released into the cytoplasm. The viral nucleocapsid is transported to the nucleus, where it docks to a nuclear pore and the genome DNA enters the nucleus along with tegu-ment protein VP16. The linear genome DNA now becomes circular. VP16 interacts with cellular transcription factors to activate transcription of viral immediate early (IE) genes by host cell RNA polymerase. IE mRNA is translated into I proteins that regulate the synthesis of early proteins such as the DNA polymerase that replicates the genome and thymidine kinase. These two proteins are important because they are involved in the action of acyclovir, which is the most important drug effective against HSV.

Note that early protein synthesis by HSV can be subdivided into two categories: immediate early and early. Immediate early proteins are those whose mRNA synthesis is activated by a protein brought in by the incoming parental virion (i.e., no new viral protein synthesis is required for the production of the five immediate early proteins. The early proteins, on the other hand, do require the synthesis of new viral regulatory proteins to activate the transcription of their mRNAs.

The viral DNA polymerase replicates the genome DNA, at which time early protein synthesis is shut off and late protein synthesis begins. These late, structural proteins are transported to the nucleus, where virion assembly occurs.

The virion obtains its envelope by budding through the nuclear membrane and exits the cell via tubules or vacuoles that communicate with the exterior.

***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 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 reinfec-tion and reactivation occur in the presence of circulating IgG. Cell-mediated immunity is important in limiting herpesviruses, because its suppression often results in reac-tivation, spread, and severe disease.

***Clinical Findings***

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

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 . Recurrences frequently reappear at the same site.Keratoconjunctivitis is characterized by corneal ulcers and lesions of the conjunctival epithelium.

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.

Many infections are asymptomatic (i.e., many people have antibody to HSV-2 but have no history of disease).

Asymptomatic infections occur in both men (in the prostate or urethra) and women (in the cervix). Shedding of virus from those with an asymptomatic infection occurs sporadically and can be a source of infection of other individuals.

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.



Herpes labialis

***Laboratory Diagnosis***

An important diagnostic procedure is isolation of the virus from the lesion by growth in cell culture. The typical cyto-pathic 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 (see Figure 37-2).

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 assav. 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.

***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; fos-carnet can be used in these cases. 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 acy-clovir, 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 (shin-gles) 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; more than 90% of people in the United States have antibody by age 10 years. Varicella occurs worldwide. Prior to 2001, there were more cases of chickenpox than any other notifiable disease, but the widespread use of the vaccine has significantly reduced the number of cases. 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 immunocompro-mised 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.

***Clinical Findings***

Varicella

After an incubation period of 14 to 21 days, brief prodro-mal symptoms of fever and malaise occur. A papulovesicu-lar 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.

Zoster

The occurrence of painful vesicles along the course of a sensory nerve of the head or trunk is the usual picture . The pain can last for weeks, and post-zoster neuralgia (also known as postherpetic neuralgia) can be debilitating. In immunocompromised patients, life-threatening disseminated infections such as pneumonia can occur.

***Laboratory Diagnosis***

Although most diagnoses are made clinically, laboratory tests are available. A presumptive diagnosis can be made by using the Tzanck smear. Multinucleated giant cells are seen in VZV as well as HSV lesions (see Figure 37-2). The definitive diagnosis is made by isolation of the virus in cell culture and identification with specific antiserum. A rise in antibody titer can be used to diagnose varicella but is less useful in the diagnosis of zoster.

***Treatment***

No antiviral therapy is necessary for chickenpox or zoster in immunocompetent children. Immunocompetent adults with either moderate or severe cases of chickenpox or

zoster often are treated with acyclovir because it can reduce the duration and severity of symptoms. Immunocompro-mised children and adults with chickenpox, zoster, or disseminated disease should be treated with acyclovir. Disease caused by acyclovir-resistant strains of VZV can be treated with foscarnet. Two drugs similar to acyclovir, famciclovir (Famvir) and valacyclovir (Valtrex), can be used in patients with zoster to accelerate healing of the lesions, but none of these drugs can cure the latent state. There is some evidence that these drugs reduce the incidence of postzoster neuralgia.

***Prevention***

There are two vaccines against VZV: one designed to prevent varicella, called Varivax, and the other designed to prevent zoster, called Zostavax. Both contain live, attenuated VZV, but the zoster vaccine contains 14 times more virus than the varicella vaccine.

*CYTOMEGALOVIRUS (CMV)*

***Diseases***

CMV causes cytomegalic inclusion disease (especially congenital abnormalities) in neonates. It is the most common cause of congenital abnormalities in the United States.

CMV is a very important cause of pneumonia and other diseases in immunocompromised patients such as recipients of bone marrow (stem cell) and solid organ trans-plants. It also causes heterophil-negative mononucleosis in immunocompetent individuals.

***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.

Summary of Replicative Cycle

The cycle is similar to that of HSV (see page 290). One unique feature of CMV replication is that some of its "immediate early proteins" are translated from mRNAs brought into the infected cell by the parental virion rather than being translated from mRNAs synthesized in the newly infected cell.

***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 asvmp-tomatic, except in immunocompromised individuals. CMV enters a latent state primarily in monocytes and can be reactivated when cell-mediated immunity is decreased.

**Clinical Findings**

Approximately 20% of infants infected with CMV during gestation show clinically apparent manifestations of cyto-megalic inclusion disease such as microcephaly, seizures, deafness, jaundice, and purpura. The purpuric lesions resemble a "blueberry muffin" and are due to thrombocy-topenia. Hepatosplenomegaly is very common. Cytome-galic inclusion disease is one of the leading causes of mental retardation in the United States. Infected infants can continue to excrete CMV, especially in the urine, for several years.

In immunocompetent adults, CMV can cause hetero-phil-negative mononucleosis, which is characterized by fever, lethargy, and the presence of abnormal lymphocytes in peripheral blood smears. In immunocompromised patients, systemic CMV infections, especially pneumoni-tis, esophagitis, and hepatitis, occur in a high proportion of those 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 immunofluores cent 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 anti- body 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 orbody fluids, such as spinal fluid and amniotic fluid, are also very useful.

***Treatment***

Ganciclovir (Cytovene) is moderately effective in the treatment of CMV retinitis and pneumonia in patients with AIDS. Valganciclovir, which can be taken orally, is also effective against CMV retinitis. CMV strains resistant to ganciclovir and valganciclovir have emerged, mostly due to mutations in the d gene that encodes the phosphokinase. Drug susceptibility testing can be done.

Foscarnet (Foscavir) is also effective but causes more side effects. Unlike HS and VZV, CMV is largely resistant to acyclovir. Cidofovir (Vistide) is also useful in the treatment of CMV retinitis. Fomivirsen (Vitravene) is an antisense DNA approved for the intraocular treatment of CMV retini-tis. It is the first and, at present, the only antisense molecule to be approved for the treatment of human disease.

***Prevention***

There is no vaccine. Ganciclovir can suppress progressive retinitis in AIDS patients. Infants with cytomegalic inclusion disease who are shedding virus in their urine should be kept isolated from other infants. Blood for transfusion to newborns should be CMV antibody-negative. If possible, only organs from CMV antibody-negative donors should be transplanted to antibody-negative recipients. A high-titer immune globulin preparation (CytoGam) is used to prevent disseminated CMV infections in organ transplant patients.

*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 lvm-phoid cells, primarily B lymphocytes. EBV also infects the epithelial cells of the pharynx, resulting in the prominent sore throat. In latently infected cells, EB 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 is translated into protein.

***Transmission & Epidemiology***

EBV is transmitted primarily by the exchange of saliva (e.g., during kissing). The saliva of people with a reactiva-tion 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.

EBV infection is one of the most common infections worldwide; more than 90% of adults in the United States have antibody. Infection in the first few years of life is usually asymptomatic. Early infection tends to occur in individuals in lower socioeconomic groups. The frequency of clinically apparent infectious mononucleosis, however, is highest in those who are exposed to the virus later in life

***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 hetero-phil 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, EB V 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.

***Laboratory Diagnosis***

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

In the hematologic approach, absolute lymphocyto-sis 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.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.

***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.

***Prevention***

There is no EBV vaccine.

HUMAN HERPESVIRUS 8 (KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS)

In 1994, it was reported that a new herpesvirus, now known as human herpesvirus 8 (HHV-8), or Kaposi's sar-coma-associated herpesvirus (KSHV), causes Kaposis 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. On DNA analysis, HHV-8 resembles the lymphotropic herpesviruses (e.g., EBV and herpesvirus saimiri) more than it does the neurotropic herpesviruses, such as HSV and VZV.

Additional support was provided by serologic studies showing that most HIV-infected patients with KS had antibodies to HHV-8, whereas considerably fewer HIV- infected patients without KS had antibodies to the virus, and very few patients with other sexually transmitted diseases, but who were not HIV-infected, had these antibodies.

**PICORNAVIRUSES**

Picornaviruses are small (20-30 nm) nonenveloped viruses composed of an icosahedral nucleocapsid and a single-stranded RNA genome. The genome RNA has positive polarity (i.e., on entering the cell, it functions as the viral mRNA). There is no polymerase within the virion. Picor-naviruses replicate in the cytoplasm of cells. They are not inactivated by lipid solvents, such as ether, because they do not have an envelope.

The picornavirus family includes two groups of medical importance: the enteroviruses and the rhinoviruses.

Among the major enteroviruses are poliovirus, Coxsackie viruses, echoviruses, and hepatitis A virus . Enteroviruses infect primarily the enteric tract, whereas rhinoviruses are found in the

nose and throat

Enteroviruses replicate optimally at 37°C, whereas rhinoviruses grow better at 33°C, in accordance with the lower temperature of the nose. Enteroviruses are stable under acid conditions (pH 3-5), which enables them to survive exposure to gastric acid, whereas rhinoviruses are acid-labile. This explains why rhinovirus infections are restricted to the nose and throat.

ENTEROVIRUSES

1. Poliovirus

***Disease***

This virus causes poliomyelitis.

Important Properties

The host range is limited to primates (ie., humans and nonhuman primates such as apes and monkeys). This limitation is due to the binding of the viral capsid protein to a receptor found only on primate cell membranes. However, note that purified viral RNA (without the capsid protein) can enter and replicate in many nonprimate cells--the RNA can bypass the cell membrane receptor (i.e., it is "infectious RNA"). There are three serologic (antigenic) types based on different antigenic determinants on the outer capsid pro-teins. Because there is little cross-reaction, protection from disease requires the presence of antibody against each of the three types.

Summary of Replicative Cycle

The virion interacts with specific cell receptors on the cell membrane and then enters the cell. The capsid proteins are then removed. After uncoating, the genome RNA functions as mRNA and is translated into one very large polypeptide called noncapsid viral protein 00. This polypeptide is cleaved by a virus-encoded protease in multiple steps to form both the capsid proteins of the progeny virions and several noncapsid proteins, including the RNA polymerase that synthesizes the progeny RNA genomes. Replication of the genome occurs by synthesis of a complementary negative strand, which then serves as the template for the positive strands. Some of these positive strands function as mRNA to make more viral proteins, and the remainder become progeny virion genome RNA.

***Transmission & Epidemiology***

Poliovirus is transmitted by the fecal-oral route. It replicates in the oropharynx and intestinal tract. Humans are the only natural hosts.

As a result of the success of the vaccine, poliomyelitis caused by naturally occurring "wild-type" virus has been eradicated from the United States and, indeed, from the entire Western hemisphere. The rare cases in the United States occur mainly in (1) people exposed to virulent revertants of the attenuated virus in the live vaccine and

(2) unimmunized people exposed to wild-type poliovirus while traveling abroad. Before the vaccine was available, epidemics occurred in the summer and fall.

***Pathogenesis & Immunity***

After replicating in the oropharynx and small intestine, especially in lymphoid tissue, the virus spreads through the bloodstream to the central nervous system. It can also spread retrograde along nerve axons.In the central nervous system, poliovirus preferentially replicates in the motor neurons located in the anterior horn of the spinal cord. Death of these cells results in

paralysis of the muscles innervated by those neurons.

Paralysis is not due to virus infection of muscle cells. The virus also affects the brainstem, leading to "bulbar" poliomyelitis (with respiratory paralysis), but rarely damages the cerebral cortex.In infected individuals, the immune response consists of both intestinal IgA and humoral IgG to the specific sero-type. Infection provides lifelong type-specific immunity.

***Clinical Findings***

The range of responses to poliovirus infection includes (1) inapparent, asymptomatic infection; (2) abortive polio-myelitis; (3) nonparalytic poliomyelitis; and (4) paralytic poliomyelitis. Asymptomatic infection is quite common. Roughly 1% of infections are clinically apparent. The incubation period is usually 10 to 14 days. The most common clinical form is abortive poliomyelitis, which is a mild, febrile illness characterized by headache, sore throat, nausea, and vomiting. Most patients recover spontaneously. Nonparalytic poliomyelitis manifests as aseptic meningitis with fever, headache, and a stiff neck. This also usually resolves spontaneously. In paralytic poliomyelitis, flaccid paralysis is the predominant finding, but brainstem involvement can lead to life-threatening respiratory paraly-sis. Painful muscle spasms also occur. The motor nerve damage is permanent, but some recovery of muscle function occurs as other nerve cells take over. In paralytic polio, both the meninges and the brain parenchyma (meningoencepha-litis) are often involved. If the spinal cord is also involved, the term meningomyeloencephalitis is often used.

***Laboratory Diagnosis***

The diagnosis is made either by isolation of the virus or by a rise in antibody titer. Virus can be recovered from the throat, stool, or spinal fluid by inoculation of cell cultures.

The virus causes a cytopathic effect (CPE) and can be identified by neutralization of the CPE with specific antisera.

***Treatment***

There is no antiviral therapy. Treatment is limited to symptomatic relief and respiratory support, if needed. Physiotherapy for the affected muscles is important.

***Prevention***

Poliomyelitis can be prevented by both the killed vaccine (Salk vaccine, inactivated vaccine, IPV) and the live, attenuated vaccine (Sabin vaccine, oral vaccine, OPV) Both vaccines induce humoral antibodies, which neutralize virus entering the blood and hence prevent central nervous system infection and disease. Both the killed and the live vaccines contain all three serotypes.

At present, the inactivated vaccine is preferred for reasons that are described later.

The current version of the inactivated vaccine is called enhanced polio vaccine, or eIPV. It has a higher seroconversion rate and induces a higher titer of antibody than the previous IPV.

2. Coxsackie Viruses

Coxsackie viruses are named for the town of Coxsackie, NY, where they were first isolated.

***Diseases***

Coxsackie viruses cause a variety of diseases. Group A viruses cause, for example, herpangina, acute hemorrhagic conjunctivitis, and hand-foot-and-mouth disease, whereas group B viruses cause pleurodynia, myocarditis, and peri-carditis. Both types cause nonspecific upper respiratory tract disease (common cold), febrile rashes, and aseptic meningitis. Coxsackie viruses and echoviruses (see next section) together cause approximately 90% of cases of viral (aseptic) meningitis.

***Important Properties***

The size and structure of the virion and the nature of the genome RNA are similar to those of poliovirus. The classification of Coxsackie viruses into group A or B is based on pathogenicity in mice. Group A viruses cause widespread myositis and flaccid paralysis, which is rapidly fatal, whereas group B viruses cause generalized, less severe lesions of the heart, pancreas, and central nervous system and focal myositis. At least 24 serotypes of Coxsackie virus A and 6 serotypes of Coxsackie virus B are recognized.

***Transmission & Epidemiology***

Coxsackie viruses are transmitted primarily by the fecal-oral route, but respiratory aerosols also play a role. They replicate in the oropharynx and the intestinal tract. Humans are the only natural hosts. Coxsackie virus infections occur worldwide, primarily in the summer and fall.

***Pathogenesis & Immunity***

Group A viruses have a predilection for skin and mucous membranes, whereas group B viruses cause disease in various organs such as the heart, pleura, pancreas, and liver. Both group A and B viruses can affect the meninges and the motor neurons (anterior horn cells) to cause paralysis.

From their original site of replication in the oropharynx and gastrointestinal tract, they disseminate via the bloodstream. Immunity following infection is provided by type-specific IgG antibody.

***Clinical Findings***

Group A-Specific Diseases

Herpangina is characterized by fever, sore throat, and tender vesicles in the oropharynx. Hand-foot-and-mouth disease is characterized by a vesicular rash on the hands and feet and ulcerations in the mouth, mainly in children. Group B-Specific Diseases Pleurodynia (Bornholm disease, epidemic myalgia, "devil's grip") is characterized by fever and severe pleuritic-type chest pain. Note that pleurodynia is pain due to an infection of the intercostal muscles (myositis), not of the pleura. Myocarditis and pericarditis are characterized by fever, chest pain, and signs of congestive failure. Dilated cardiomyopathy with global hypokinesia of the myocardium is a feared sequela that often requires cardiac transplantation to sustain life. Diabetes in mice can be caused by pancreatic damage as a result of infection with Coxsackie virus B4.

This virus is suspected to have a similar role in juvenile diabetes in humans. Diseases Caused by Both Groups Both groups of viruses can cause aseptic meningitis, mild paresis, and acute flaccid paralysis similar to poliomyelitis. Upper respiratory infections, pharyngitis, and minor febrile illnesses with or without rash can occur also.

***Laboratory Diagnosis***

The diagnosis is made either by isolating the virus in cell culture or suckling mice or by observing a rise in titer of neutralizing antibodies. A rapid (2.5-hour) polymerase chain reaction (PCR)-based test for enteroviral RNA in the spinal fluid is useful for making a prompt diagnosis of viral meningitis because culture techniques typically take days to obtain a result.

***Treatment & Prevention***

There is neither antiviral drug therapy nor a vaccine available against these viruses. No passive immunization is recommended.

3. Echoviruses

The prefix ECHO is an acronym for enteric cytopathic human orphan. Although called "orphans" because they were not initially associated with any disease, they are now known to cause a variety of diseases such as aseptic menin-gitis, upper respiratory tract infection, febrile illness with and without rash, infantile diarrhea, and hemorrhagic conjunctivitis.

The structure of echoviruses is similar to that of other enteroviruses. More than 30 serotypes have been isolated. In contrast to Coxsackie viruses, they are not pathogenic for mice. Unlike polioviruses, they do not cause disease in monkeys. They are transmitted by the fecal-oral route and occur worldwide. Pathogenesis is similar to that of the other enteroviruses.

Along with Coxsackie viruses, echoviruses are one of the leading causes of aseptic (viral) meningitis. The diagnosis is made by isolation of the virus in cell culture.

Serologic tests are of little value, because there are a large number of serotypes and no common antigen. There is no antiviral therapy or vaccine available.

**RHABDOVIRUSES**

RABIES VIRUS

***Disease***

This virus causes rabies, an encephalitis.

***Important Properties***

Rabies virus is the only medically important member of the rabdovirus family. It has a single-stranded RNA enclosed within a bullet-shaped capsid surrounded by a lipoprotein envelope. Because the genome RNA has negative polarity, the virion contains an RNA-dependent RNA polymerase. Rabies virus has a single antigenic type. The antigenicity resides in the envelope glycoprotein spikes. Rabies virus has a broad host range: It can infect all mammals, but only certain mammals are important sources of infection for humans (see later.

Summary of Replicative Cycle

Rabies virus attaches to the acetylcholine receptor on the cell surface. After entry into the cell, the virion RNA polymerase synthesizes five mRNAs that code for viral proteins.

After replication of the genome viral RNA by a virus-encoded RNA polymerase, progeny RNA is assembled with virion proteins to form the nucleocapsid, and the envelope is acquired as the virion buds through the cell membrane.

***Transmission & Epidemiology***

The virus is transmitted by the bite of a rabid animal that manifests aggressive, biting behavior induced by the viral encephalitis. The virus is in the saliva of the rabid animal.

In the United States, transmission is usually from the bite of wild animals such as skunks, raccoons, and bats; dogs and cats are frequently immunized and therefore are rarely sources of human infection. In recent years, bats have been the source of most cases of human rabies in the United States. Rodents and rabbits do not transmit rabies. Human rabies has also occurred in the United States in people who have not been bitten, so-called "nonbite" expo-sures. The most important example of this type of transmission is exposure to aerosols of bat secretions containing rabies virus. Another rare example is transmission in transplants of corneas taken from patients who died of undiagnosed rabies.

***Pathogenesis & Immunity***

The virus multiplies locally at the bite site, infects the sensory neurons, and moves by axonal transport to the central nervous system. During its transport within the nerve, the virus is sheltered from the immune system and little, if any, immune response occurs. The virus multiplies in the central nervous system and then travels down the peripheral nerves to the salivary glands and other organs. From the salivary glands, it enters the saliva to be transmitted by the bite. There is no viremic stage. Within the central nervous system, encephalitis devel-ops, with the death of neurons and demyelination. Infected neurons contain an eosinophilic cytoplasmic inclusion called a Negri body, which is important in laboratory diagnosis of rabies . Because so few individuals have survived rabies, there is no information regarding immunity to disease upon being bitten again.

***Clinical Findings***

The incubation period varies, according to the location of the bite, from as short as 2 weeks to 16 weeks or longer. It is shorter when bites are sustained on the head rather than on the leg, because the virus has a shorter distance to travel to reach the central nervous system.

Clinically, the patient exhibits a prodrome of nonspecific symptoms such as fever, anorexia, and changes insensation at the bite site called paresthesias. After the pro-drome, rabies manifests as either of two forms: "furious" (encephalitic) or "dumb" (paralytic). The furious form occurs in about 80% of cases. In the furious form, agitation, delirium, seizures, and hydrophobia occur.

***Laboratory Diagnosis***

Rapid diagnosis of rabies infection in the animal is usually made by examination of brain tissue by using either PCR assay, fluorescent antibody to rabies virus, or histologic staining of Negri bodies in the cytoplasm of hippocampal neurons (see Figure 39-6). The virus can be isolated from the animal brain by growth in cell culture, but this takes too long to be useful in the decision of whether to give the vaccine. Rabies in humans can be diagnosed by PCR assay; by fluorescent antibody staining of a biopsy specimen, usually taken from the skin of the neck at the hairline; by isolation of the virus from sources such as saliva, spinal fluid, and brain tissue; or by a rise in titer of antibody to the virus. Negri bodies can be demonstrated in corneal scrapings and in autopsy specimens of the brain.

***Treatment***

There is no antiviral therapy for a patient with rabies. Only supportive treatment is available.

***Prevention***

In the United States, the rabies vaccine contains inactivated virus grown in human diploid cells. (Vaccine grown in monkey lung cells or chick embryo cells is also available.) In other countries, the duck embryo vaccine or various nerve tissue vaccines are available as well. Duck embryo vaccine has low immunogenicity, and the nerve tissue vaccines can cause an allergic encephalomyelitis as a result of a cross-reaction with human myelin. For these reasons, the human diploid cell vaccine (HDCV) is preferred.

**ARBOVIRUSES**

INTRODUCTION

Arbovirus is an acronym for arthropod-borne virus and highlights the fact that these viruses are transmitted by arthropods, primarily mosquitoes and ticks. It is a collective name for a large group of diverse viruses, more than 600 atlast count. In general, they are named either for the diseases they cause (e.g., yellow fever virus) or for the place where they were first isolated (e.g., St. Louis encephalitis virus). A new group of viruses called roboviruses has recently

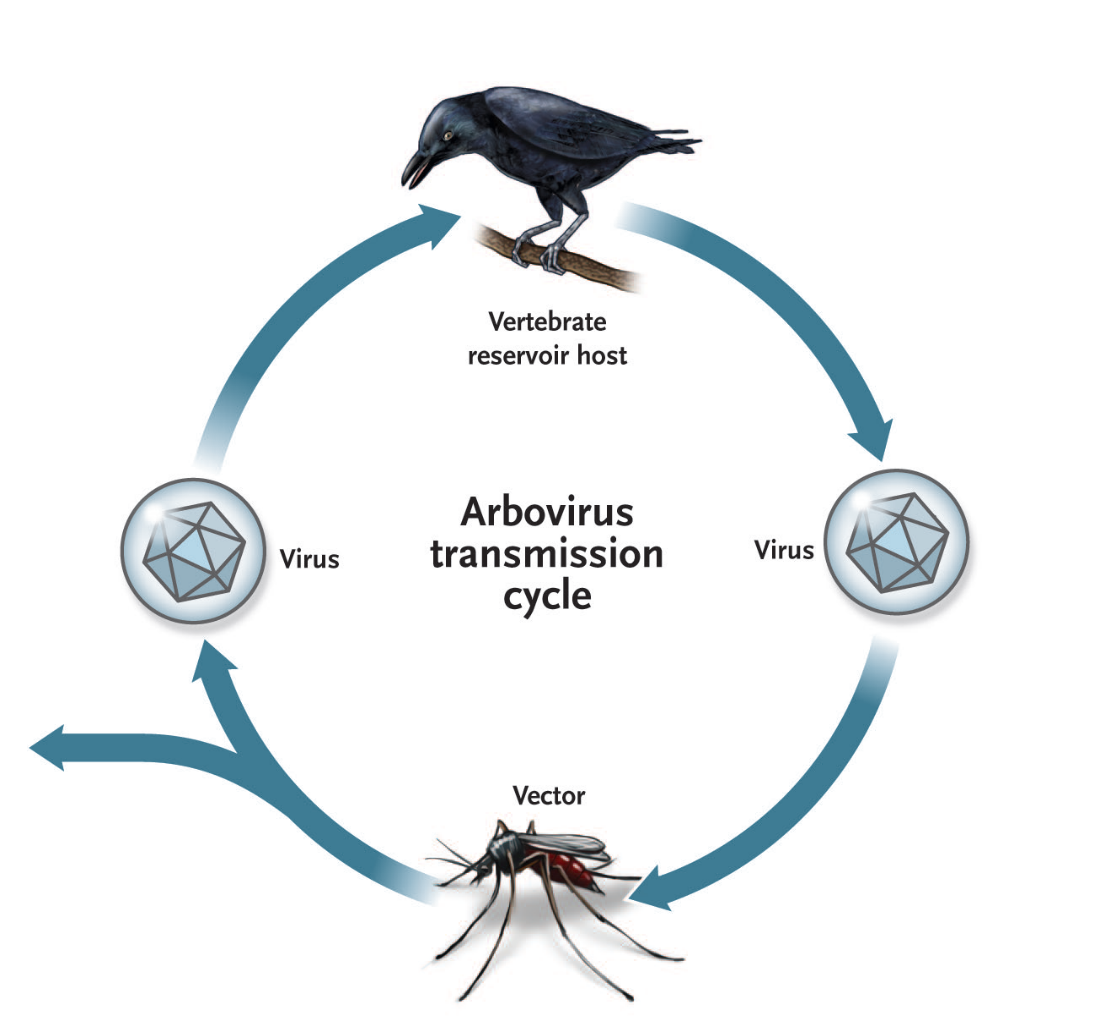
emerged. The term robo refers to the fact that these viruses are rodent-borne (i.e., they are transmitted directly from rodents to humans without an arthropod vector). Transmission

occurs when dried rodent excrement is inhaled into the human lung, as when sweeping the floor of a cabin. Two roboviruses cause a respiratory distress syndrome that is often fatal: Sin Nombre virus (a hantavirus) and Whitewater Arroyo virus (an arenavirus).

***Important Properties***

Most arboviruses are classified in three families,1 namely, togaviruses, flaviviruses, and bunyaviruses (1) Togaviruses2 are characterized by an icosahedral nucleocapsid surrounded by an envelope and a singlestranded, positive-polarity RNA genome. They are 70 nm in diameter, in contrast to the flaviviruses, which are 40 to 50 nm in diameter (see later). Togaviruses are divided into two families, alphaviruses and rubiviruses. Only alphaviruses are considered here. (2) Flaviviruses3 are similar to togaviruses in that they also have an icosahedral nucleocapsid surrounded by an envelope and a single-stranded, positive-polarity RNA genome, but the flaviviruses are only 40 to 50 nm in diameter, whereas the togaviruses have a diameter of 70 nm.

(3) Bunyaviruses4 have a helical nucleocapsid surrounded by an envelope and a genome consisting of three segments of negative-polarity RNA that are hydrogenbonded together.



***Transmission***

The life cycle of the arboviruses is based on the ability of these viruses to multiply in both the vertebrate host and the bloodsucking vector. For effective transmission to occur, the virus must be present in the bloodstream of the vertebrate host (viremia) in sufficiently

high titer to be taken up in the small volume of blood ingested during an insect bite. After ingestion, the virus replicates in the gut of the arthropod and then spreads to other organs, including the salivary glands. Only the female of the species serves as the vector of the

virus, because only she requires a blood meal in order for progeny to be produced. An obligatory length of time, called the extrinsic incubation period,5 must pass before the virus has replicated sufficiently for the saliva of the vector to contain enough virus to transmit an infectious dose. For most viruses, the extrinsic incubation period ranges from 7 to 14 days. In addition to transmission through vertebrates, some arboviruses are transmitted by vertical “transovarian” passage from the mother tick to her offspring. Vertical transmission has important survival value for the virus if a vertebrate host is unavailable. Humans are involved in the transmission cycle of arboviruses in two different ways. Usually, humans are deadend hosts, because the concentration of virus in human blood is too low and the duration of viremia is too brief for

the next bite to transmit the virus. However, in some diseases (e.g., yellow fever and dengue), humans have a highlevel viremia and act as reservoirs of the virus. Infection by arboviruses usually does not result in disease either in the arthropod vector or in the vertebrate

animal that serves as the natural host. Disease occurs primarily when the virus infects dead-end hosts. For example, yellow fever virus cycles harmlessly among the jungle monkeys in South America, but when the virus infects a human, yellow fever can occur.

***Clinical Findings & Epidemiology***

Most human arboviral infections are asymptomatic. Of those infections that are symptomatic, most are acute febrile illnesses. A minority of infections cause neuroinvasive disease, such as encephalitis and meningitis. The diseases caused by arboviruses range in severity from mild to

rapidly fatal. The clinical picture usually fits one of three categories: (1) encephalitis; (2) hemorrhagic fever; or (3) fever with myalgias, arthralgias, and nonhemorrhagic rash. The pathogenesis of these diseases involves not only the cytocidal effect of the virus, but also, in some, a prominent immunopathologic component. After recovery from the disease, immunity is usually lifelong. The arboviral diseases occur primarily in the tropics but are also found in temperate zones such as the United States and as far north as Alaska and Siberia. They have a tendency to cause sudden outbreaks of disease, generally at the interface between human communities and jungle or forest areas.