**Herpesviruses**

**Note:** Syllabus notes refer to the slides for Dr. Gershon’s lecture, which may be downloaded from the course website.

**Slide 1**

Common features of the herpesviruses are:

1. similar morphology
2. similar modes of replication
3. ability to cause a **primary** or first infection, followed by **latent** infection that is lifelong for the host
4. ubiquitous and thus found all over the world

Herpesviruses have two basic forms of replication, lytic and latent. During lytic infection, all of the genes of the virus are expressed, and as a result, the host cell dies. During latent infection, there is limited gene expression, and the host cell survives, in some cases for many years. During latent infection, the host has no symptoms.

It is possible for latency to be overcome, leading to lytic infection. Often, this process is accompanied by symptoms in the host. A well known example is when herpes simplex virus (HSV) type 1 causes a primary infection in the mouth (gingivostomatitis) followed by latency. Years later, the latent (limited gene expression) HSV can reactivate (express all of its genes) to cause a cold sore at the corner of the mouth, which can be painful, annoying, and enables the virus to spread to another person.

Herpesviruses can cause re-infections, because immunity to these viruses is often incomplete. Thus although there is only 1 antigenic type of HSV -1, individuals who have been infected with HSV previously can be re-infected with a slightly different HSV-1.

After primary infection or reactivated latent infection, low grade virus multiplication rarely can occur, often with some symptoms. This is called persistent infection and is a form of lytic replication.

**Slide 2**

There are 8 known Herpesviruses that infect humans (many more infect animals but not people). They are divided into alpha, beta, gamma viruses.

**Overview.**

**Alphaviruses** include HSV types 1 and 2, and varicella-zoster virus (VZV).
Alphaviruses have a short replication cycle, a variable host range, and become latent in sensory neurons. HSV causes a variety of infections of the skin and mucous membranes, genitalia, and nervous system. VZV causes chickenpox and shingles (zoster).

**Beta herpesviruses** include cytomegalovirus (CMV) and human herpesviruses 6 and 7 (HHV 6 and HHV 7). These viruses have a long replication cycle, a narrow host range, and become latent in lymphoid and other cells (such as salivary glands and kidney). They cause systemic infections that are often asymptomatic in otherwise healthy people. They also cause severe infections in the fetus and in immunocompromised patients.

**Gamma herpesviruses** include Epstein Barr Virus (EBV) and human herpes virus 8 (HHV 8) or Kaposi sarcoma virus (KSV). These viruses have a narrow host range, become latent in lymphoid cells, and are associated with tumors. EBV was discovered in the 1970s, and causes infectious mononucleosis (known as “mono” by college students) and a variety of tumors.

**Slide 3**

**Transcription of viral genome**

This process is similar for all of the herpesviruses. Replication is associated with expression of viral genes and progresses as a cascade in 3 stages, known as immediate early (IE), early (E), and late (L), leading to lytic infection.

Latency may involve a break that occurs between the first and second stages of viral gene expression. The cause of the interruption of the cascade is unknown and is the subject of intense research.

IE genes translate proteins that bind DNA and also regulate the expression of other genes. E genes encode for proteins that are transcription factors and enzymes such as DNA polymerase, and thymidine kinase (TK), that are crucial for viral replication. (As will be seen, some of these enzymes are excellent targets for antiviral therapy.) L genes encode for structural proteins, such as the glycoproteins of the virus which form its envelope or coat. In general the envelope is required for infectivity of the virus.

**Slide 4**

This slide depicts the typical appearance of a herpesvirus. It is composed of a phospho-glycolipid envelope, a tegument, an icosahedral (20-sided) capsid, and a DNA core. This virus is VZV, from the skin of a patient with chickenpox, but all of the other herpesviruses are indistinguishable from it, as seen by electron microscopy.
VZV is seen in this diagram as a prototype of herpesvirus structure. The envelope contains glycoproteins (gps), designated: gB, gC, gE etc. These appear not only on the surface of the virion (virus particle), but also on the surface of the infected host cell. The gps play an important role in spreading infection from one cell to another cell, and they are a significant target for host immunity. Herpesviruses can spread in two ways, as the free enveloped particle, and also from cell-to-cell. For the latter type of spread, cellular immunity of the host is critical in defense. Antibodies cannot reach viruses multiplying within cells. The herpesviruses utilize a great deal of cell-to-cell spread, which is why cellular immunity is crucial for host defense against this virus.

The tegument contains proteins of open reading frames (ORFs) numbers 4, 10, 21, 62, and 63. ORF 62 is an important IE gene which is critical for formation of new viruses and also maintenance of latent infection, and is a lynchpin for synthesis of VZV.

This slide shows a diagram of the organization of the VZV genome. Similar diagrams have been developed for the other human herpesviruses. The genome is divided into unique long and short sequences, which contain internal and external repeats.
- U = unique
- L = long
- S = short
- T = terminal
- R = repeat

These electron micrographs depict formation of a herpesvirus in an infected cell, using VZV as a prototype.

Upper left corner: DNA replicates in the nucleus and nucleocapsids are formed there, which are extruded into the perinuclear space. An envelope can be seen surrounding the capsid, but this is not the final envelope.

Upper right corner
A nucleocapsid with an early envelope progresses to cytosol, where it is extruded as its envelope is fused with rough endoplasmic reticulum (RER), releasing the naked nucleocapsid into the cytosol.
Glycoproteins are made in the RER and transported to the Golgi. Tegument proteins are made in the cytosol and are also transported to the Golgi. In the trans Golgi network (TGN), due to processes of protein signaling, capsid, tegument, and glycoproteins all come together. Nucleocapsids are then enveloped by the tegument proteins and glycoproteins, leading to enveloped virions, as seen in the lower right corner.

These electron micrographs show further details concerning envelopment of VZV, as just described.

This diagram shows how VZV receives its final envelope in the TGN. Because the gps of VZV contain mannose 6-phosphate, the virions are incorporated into endosomes, where they are inactivated by the acid environment and are not released extracellularly in infectious form. This is true when VZV is propagated in cell culture in the laboratory and also in the infected host, where the virus spreads only from cell to cell. Therefore in host defense, VZV must be attacked with a cell-mediated response and the antibody response is not helpful.

HSV has an unknown means whereby it is able to evade endosomes and as such is released extracellularly in infectious form. Although the primary form of host defense against HSV is cellular immunity, antibodies may play more of a role in defense than for VZV infection.

For HSV as for many viruses, enveloped viral particles invade cells by utilizing receptors on the cell surface. Often, more than one receptor may be used. Receptors for HSV include heparan sulfate, and Hve A-C and Nectin-1, and 2 which are members of the immunoglobulin and tumor necrosis factor (TNF) families. Receptors for VZV include heparan sulfate and the mannose 6-phosphate receptor.

Glycoproteins B, D, H and L on the virion enable it to attach to cells and infect them. Recall that these gps are also present on the surface of infected cells, so another mode of spread for HSV is by cell to cell contact, utilizing the same gps to attach to the cell and invade it. Interestingly, although gD is the major gp of HSV, VZV lacks gD and probably substitutes gE for infectivity. gE is a minor gp of HSV, although it is the major one for VZV. Glycoproteins are components of a new, experimental HSV2 vaccine, which stimulates immunity that prevents the virus from carrying out these functions, i.e. spreading into a new cell to infect it.
Lytic infection with HSV results in cell death; latent infection allows cell to live (unless reactivation occurs, at which point the cell dies). Latency for HSV is different than for VZV. With HSV a very limited repertoire of gene expression occurs; only a few so-called latency associated transcripts (LATS) are expressed, and there is no protein expression. Thus there is minimal transcription of DNA, and no translation of proteins in latency of HSV. Again, what controls this process is unknown.

**Slide 11**

HSV 1 infections are complex. They are classified as primary, non-primary, first episode, recurrent, and reinfection. Many HSV infections are asymptomatic, which further accounts for the complexity of definitions.

**Primary infection** with HSV 1 is very common and usually occurs in first few years of life. It may be symptomatic, as gingivostomatitis (picture follows) or asymptomatic.

Primary genital HSV 2 infection is not universal as is HSV 1 infection, although it is becoming more common. It typically occurs after the first decade of life, when sexual activity begins. Because it can be transmitted from a pregnant woman to her baby, it is also seen in newborn infants, in whom it is often severe or fatal, if untreated.

**Non-primary infection** is defined as first infection with one HSV in the setting of past infection with the other. Usually this means HSV 2 infection in someone who was already infected with HSV 1. Alternatively someone who is first infected with HSV 2 may then be infected by HSV 1 which would be called non-primary. Non-primary infections tend to be milder than primary infections.

**First episode disease** is present when an individual has a first HSV infection that is asymptomatic but then develops a symptomatic infection, either due to reactivation of the first HSV, or reinfection with a new one.

**Recurrent infections** are due to reactivation of latent infection. Typically for HSV 1, painful vesicles or ulcers appear at the corners of the mouth. Areas of the skin may also be involved; clustered vesicles are classic for this presentation. For HSV 1, infections usually occur above the belt, commonly on the face, but also on the trunk. For HSV 2, infections usually occur below the belt. Genital HSV 2 may be primary, non-primary, first episode, or recurrent.

Recurrent infections are provoked by a number of stimuli, such as fever, trauma, sunlight, stress, and menstruation. Reactivation occurs despite the presence of specific antibodies, and may be related to a deficient gamma interferon (IF) response. Severing of cranial nerve V is known to reactivate latent HSV-1 in animals and humans.

A recurrent vesicular rash in the same area of skin, usually on the face, is diagnostic of reactivation of HSV (not VZV).
Many HSV infections are asymptomatic. Infectious virus is produced, however, and this is termed “shedding” of virus. At any one time, perhaps 3% of healthy adults shed HSV 1 with no symptoms. Individuals shedding virus are infectious to others, for both HSV 1 and for HSV 2. Asymptomatic shedding is thought to be a major means by which HSV 2 is spread sexually.

Host factors are very important in whether HSV infections are severe. The cell-mediated immune response appears to be critical. Thus severe infections are seen in the immunocompromised and newborn babies (who have immature cell-mediated immune responses). Immunocompromised patients include: individuals receiving chemo-and radio-therapy for cancer, transplant patients, patients on high steroids for any reason, and patients born with immunodeficiency diseases, particularly those with congenital defects in cell-mediated immunity.

Slide 12

There are a number of specific infections caused by HSV 1 and/or 2. These may affect skin and/or mucous membranes, occur in neonates, and involve the central nervous system (CNS).

Type 1 HSV causes gingivostomatitis, whitlow of the finger (pictures follow), keratitis (infection of the cornea of the eye), encephalitis, and severe infection of the skin in people with underlying eczema (eczema herpeticum). Neonatal infection with HSV 1 occurs but is unusual.

Type 2 HSV causes genital infection (sometimes accompanied by meningitis), and neonatal infection. HSV 2 can be passed from mother to child in the birth canal, and causes severe infections in the baby.

Disease is due to the presence of the virus growing in the cells and also from the immune response to the virus.

Slide 13

This shows a baby with primary HSV 1 gingivostomatitis. Note the ulcers on the oral mucosa, along with swollen and friable gums. There is a secondary infection of the thumb from sucking it, and there are satellite lesions on the surrounding skin. Although this infection looks very severe, it is usually self-limited. Poor nutrition and dehydration for a few days can be a problem. Many times, for unknown reasons, the primary infection can be asymptomatic, but recurrent disease (fever blisters) are symptomatic.

Primary HSV-1 is painful but causes limited problems, and patients usually are not treated with antiviral medications. It is customary to treat patients in whom genital HSV 2 is diagnosed. HSV 2 commonly recurs (more commonly than HSV 1, for unknown reasons).
N.B. the examiner must be careful to wear protective gloves when examining patients like this. In this instance the examiner was not wearing gloves and put himself at risk to develop what is depicted in the next slide.

**Slide 14**

This person has an herpetic whitlow. These lesions are painful and extremely infectious to others. Puncture of these sores produces watery fluid from which HSV (usually type 1) can be cultured. These are often confused clinically with staphylococcal infections.

**Slide 15**

HSV 1 is the cause of the most common form of focal encephalitis in US. It can usually be treated effectively if suspected early and treated promptly.

Symptoms and signs include headache, personality change, focal seizures, abnormal EEG, CT, MR. Personality change is caused by pathological involvement of the temporal lobe. Radiological findings of HSV appear late in the course of infection and they are generally not useful for early diagnosis of HSV infection. The differential diagnosis for a patient with these symptoms includes other infections as well as tumors. Diagnosis is best accomplished by performing PCR on cerebrospinal fluid (CSF). It is rare to be able to isolate HSV from CSF in encephalitis. If HSV 1 encephalitis is strongly suspected, antiviral treatment is usually begun even before the result of the PCR is known. The best prognosis is in children (rather than adults) who are treated soon after disease onset. Adults may survive, but have neurologic deficits. HSV-1 is one of the few forms of treatable encephalitis.

**Slide 16**

Perinatal HSV can be severe or fatal. It is usually due to Type 2 virus because the baby is infected from contact with the mother’s infected genital tissues and secretions if she has genital HSV at delivery. Often the mother has no symptoms of genital infection. The attack rate is > 10 times greater in maternal primary infection compared to recurrence; thus a mother who is newly infected near the time of giving birth is 10 times more likely to pass on HSV2 to her infant when giving birth vaginally. Women who have obvious genital HSV at delivery usually have a caesarean section to protect the baby from infection.

Clues to infection of the infant include skin vesicles (in 70%), fever, seizures, pneumonia, disseminated intravascular coagulation (DIC), and conjunctivitis. This is a spectrum of disease ranging from apparently mild to very severe. Therapy is aimed at preventing mild disease from progressing to severe disease.

Diagnosis is made by testing skin lesions and/or CSF for HSV 1, by culture for virus, immunofluorescence, and PCR.
Any infant in whom this diagnosis is strongly considered or proven should be treated with antiviral therapy, even if he/she is otherwise asymptomatic (i.e. has only skin vesicles). This therapy is a medical emergency and may be life saving for the infant.

In order to try to reduce vertical transmission (from mother to baby), there are hopes eventually to administer a form of HSV 2 vaccine to girls before the start of sexual activity.

Slide 17

This slide shows grouped vesicles typical of HSV on the skin of an infant who was eventually found to be infected with HSV 1. The skin lesions were positive by culture and immunofluorescence. The mother had undiagnosed HSV 1 at delivery and kissed her baby right after birth. Prompt diagnosis and treatment resulted in a normal baby. In general the prognosis of neonatal HSV 1 is better than that of HSV 2, possibly because HSV 1 is more sensitive to antiviral therapy than HSV 2.

Slide 18

This slide shows an umbilicus with tiny vesicles that were positive for HSV 2 on culture and by immunofluorescence. Early treatment with antiviral therapy leads to a very good outcome if the disease is treated before very much viral spread has taken place. Late treatment or untreated disease has a VERY poor prognosis (retardation or death).

Slide 19

Neonatal HSV has been divided into 3 clinical categories.

1. Skin and mucous membrane infection only (40% of infected babies have this form at the time of diagnosis). There are no symptoms other than the skin rash (grouped vesicles). There is an excellent prognosis with early therapy, which keeps virus from spreading. It is possible to diagnose these infections easily because the virus produces the skin lesions from which it can be identified.

2. CNS infection (35% of babies with HSV have this form at presentation). Symptoms include fever, lethargy, seizures. The CSF is abnormal. These babies often have major sequelae if they survive. The disease is hard to diagnose because the babies often don't have skin vesicles.

3. Disseminated disease (25% of infected babies present with this form). 2/3 develop skin vesicles but the 1/3 who do not can be very hard to diagnose. Signs and symptoms include hepatosplenomegaly, jaundice, hepatitis, and pneumonia. Mortality is high (70%).
Slide 20

Diagnosis of neonatal HSV can be made by: immunofluorescence, culture, and PCR. Measuring antibodies in mother and baby is not useful because it takes too long and may not yield useful data.

It is important to begin antiviral therapy as soon as possible in order to have the best outcome.

Recurrent skin vesicles in the baby are associated with a poorer prognosis.

Slide 21

VZV: varicella-zoster virus
Primary infection is varicella, a highly contagious disease spread by the airborne route. Complications of this disease, for which there is now a licensed vaccine, include bacterial superinfection, encephalitis, pneumonia, and a congenital syndrome.

Secondary infection is zoster. Zoster is due to reactivation of latent VZV; latent virus was acquired during primary infection. This has been proven because VZV DNA, RNA, and proteins have been identified at autopsy in people who never had zoster. Additionally, zoster caused by the vaccine virus (Oka strain) has been identified in a few vaccinated people. Zoster occurs when the cell-mediated immune response to VZV is low.

There is no asymptomatic shedding of VZV as there is with HSV. Zoster is infectious only when it is clinically apparent. When zoster spreads VZV to others, they develop chickenpox, not zoster.

Slide 22

This slide shows a young boy with classic varicella (chickenpox). He is not very ill, but he has skin vesicles typical of varicella. These vesicles are full of infectious virus. This is the only time that enveloped virions are produced in the body; otherwise cell to cell spread through the body is the rule. These virions can be aerosolized and thus this disease spreads by the airborne route from the skin. This boy is not very sick, which is typical for varicella. Because it is usually not possible to predict who will develop severe varicella in advance, however, the varicella vaccine was developed.
Slide 23

This slide shows a young immunocompromised adult with zoster (shingles). Classically the rash is on one side of the body and stops abruptly at the midline. This distribution of rash reflects the pathogenesis of the illness (reactivation of latent infection). Zoster occurs only in people who have had varicella previously.

Slide 24

The pathogenesis of varicella is as follows. Virions infect the respiratory mucosa and spread to T lymphocytes (viremia), where the virus is not accessible to antibodies. The virus spreads slowly from cell to cell, accounting for an incubation period of 2-3 weeks. This slow spread keeps the virus from overwhelming the host; during this time, the immune system is beginning to develop the cell-mediated response that can control the virus. In contrast to normal individuals, immunocompromised patients are deficient in this response and therefore are at risk to develop severe or fatal varicella (if they are untreated). This long incubation period is probably one reason why the varicella vaccine has been so effective in controlling varicella.

Slide 25

This slide shows an infant with the congential varicella syndrome. This baby’s mother had varicella during pregnancy and infected her fetus. The intrauterine immune response to the virus was immature, resulting in fetal infection and poorly checked viral replication. This led to overwhelming latent infection and a high degree of tissue damage. Many organ systems were affected, and this baby died before her first birthday. This is a rare outcome of maternal infection. It is likely that the problem will become even more rare because today most people are immunized against varicella.

Slide 26

This slide shows a baby whose mother had varicella just before her delivery. The baby was infected but due to immaturity could mount only a poor cell-mediated immune response. The baby had overwhelming chickenpox and died.

Slide 27
This slide shows a 3 month old baby with classic zoster. Note the distribution of the rash. The baby’s mother had varicella during pregnancy and the baby developed latent infection. The virus reactivated at an early age and produced this illness.

Slide 28

When varicella or zoster occurs in an immunocompromised patient, it can be severe or fatal. A person with no history of varicella or vaccination is termed a “susceptible”. When an immunocompromised susceptible is exposed to someone with a VZV infection, severe infection can be averted by passive immunization. Passive immunization means providing specific antibodies to modify an infection. For varicella, this is done by administering varicella-zoster immune globulin (VZIG, which has very high titers of antibody to VZV. VZIG must be given as soon as possible after exposure in order to work. It is only useful when given to a susceptible. It will not prevent reinfection and it cannot prevent zoster. People given VZIG either develop no varicella or only a mild case.

Often immunocompromised susceptible patients are unaware that they have been exposed to VZV and simply present with full-blown varicella. In this case, it is important to treat them with antiviral medications as soon as possible.

Immunocompromised patients who have had varicella are at increased risk to develop zoster, and they should receive antiviral therapy. This is probably due to defects in cell-mediated immunity. They are also more likely to develop a poorly understood pain syndrome following zoster called post-herpetic neuralgia (PHN). Persons over the age of 50 are also at increased risk to develop zoster and PHN.

Slide 29

This diagram of the skin and the sensory nerve supplying it shows the pathogenesis of latent VZV infection and zoster. During varicella the virions are present on skin and can invade the sensory neurons without multiplying (which would kill the neuron). By unknown means, the virus becomes latent. In latency, it is able to express at least 6 of its 68 genes, but the cascade of lytic infection is blocked. Subsequently, due to unknown factors, the virus reactivates in about 15% of people who have had varicella. One recently identified stimulus to VZV reactivation is an extract of stimulated mast cells containing a wide variety of chemokines and cytokines.

Slide 30

In latent VZV infection 6 regulatory proteins (from IE and E genes) are expressed. In lytic infection, this expression is nuclear, but in latent infection it is cytoplasmic. This observation suggests that the cascade of gene expression is blocked because the regulatory proteins are excluded from the nucleus in VZV
latency. In order for latent infection to occur, cell free, enveloped virus must be able to infect neurons. Latent VZV infection occurs only in neurons.

Slide 31

VZV is currently the only herpesvirus for which there is a vaccine. The vaccine is a live attenuated strain (Oka) of VZV. It was licensed for routine use in healthy persons in the US in 1995. It is a very safe vaccine and is currently being given to about 4 million American children annually. In the 8 years since licensure of the vaccine, the incidence of varicella has decreased in all age groups, indicating herd immunity. Contraindications to varicella vaccine are few: being pregnant, being allergic to vaccine components, and being immunocompromised are the only contraindications.

Slide 32

Varicella vaccine causes a mild rash about 1 month after vaccination in about 5% of those who are immunized. Spread of the vaccine virus from these skin lesions is rare and not harmful. No vaccine is 100% effective or 100% safe. Varicella vaccine protects about 85% of people from any form of the disease; the remaining 15% develop only a mild illness. There is little evidence that immunity wanes with time after vaccination. Zoster after vaccination is rare, probably because the virus rarely reaches the skin to cause latent infection.

Slide 33

This slide shows how the incidence of varicella has fallen since vaccine licensure in 1995.

Slide 34

The rash of varicella is vesicular, which facilitates rapid diagnosis, as with HSV.

Slide 35

Diseases that present with vesicles can be diagnosed rapidly by performing immunofluorescence on a smear of the vesicular fluid. This slide shows a positive and a negative (control) for HSV 1.
Slide 36

Classic methods for diagnosis of HSV and VZV include: culture, direct immunofluorescence (DFA) and PCR on smears from a skin rash. Positive cultures for HSV can develop within 24-48 hours. VZV is much more difficult to culture and results are often not available for 5-7 days.

A rise in antibody titer is also diagnostic of infection. This is performed on acute (at disease onset) and convalescent (10-14 days after onset) serum specimens, measuring specific IgG antibodies. A positive titer of specific IgM is also usually an indication of an acute infection.

Slide 37

Effective antiviral therapy has changed the prognosis of herpesvirus infections, particularly in immunocompromised patients, in the past 15 years. The most commonly employed drug is acyclovir (ACV), which interferes with viral DNA synthesis. Its mode of action is dependent on viral thymidine kinase (Tk) and DNA polymerase (E genes). Because its action depends on viral Tk, which is present only in cells infected with the virus, it is relatively non-toxic and well tolerated in patients.

HSV 1 and 2, and VZV are both sensitive to ACV, although VZV is less sensitive than HSV. HSV 1 is more sensitive than HSV 2. Patients with VZV infections are treated with higher doses of ACV than those with HSV. EBV and CMV are not sensitive to ACV. ACV can be given orally or intravenously.

Adverse effects from treatment are unusual. Gastrointestinal symptoms (in 20%) and neurologic symptoms (in 5%) (headache, anxiety, seizures, delirium) are the most common. Bone marrow suppression (anemia, low platelets [thrombocytopenia]) is associated with long-term, high dose use.

Herpesviruses can become resistant to ACV by ceasing to make Tk; this is especially likely to occur after long term use, particularly in patients with AIDS.

Newer drugs (famciclovir, valacyclovir) are administered only orally and are converted in the body to ACV. Because these newer drugs have high absorption in the gastrointestinal tract, they result in higher blood levels of ACV than can be achieved by giving ACV itself orally. Famciclovir and valacyclovir are mainly used to treat patients with genital HSV 2 and elderly patients with zoster.

Slide 38

Cytomegalovirus (CMV) is a significant cause of human disease. It is the largest of the human herpesviruses, with 208 ORFs (compare to 68 of VZV). Its major
gps are gB and gH. It also breaks the rules as a DNA virus by having mRNA in its genome. It has developed means of immune evasion, by having genes that down-regulate expression of MHC class I expression, thus interfering with responses of cytotoxic lymphocytes (CTL). Thus the virus itself is immunosuppressive. Host defense is very much dependent on cellular immunity and therefore this virus poses significant risks to the fetus and immunocompromised patients.

CMV becomes latent in bone marrow precursors of monocytes. These cells differentiate into macrophages when stimulated by antigens, reactivating their latent CMV. CMV thus is a significant threat to patients who have undergone transplantation, after which significant immune activation occurs. Reactivation of CMV can potentially occur in both the transplant recipient and in the transplanted organ.

**Slide 39**

CMV infections in healthy adult hosts are almost always subclinical. A rare mononucleosis-like syndrome is attributed to CMV, however, which is self-limited. In contrast, severe CMV infections occur in immunocompromised hosts. These opportunistic infections are particularly a problem for patients with AIDS, following various forms of transplantation and in other immunocompromised patients.

When pregnant women are infected with CMV, their fetus may be infected. Because of immaturity of the fetal immune system, the fetus may develop what is termed congenital CMV infection. In its full-blown form, this syndrome can be severe or fatal. In contrast, when a baby acquires CMV infection at birth, from contact with infected maternal secretions, the infection is innocuous. Similarly, babies infected with CMV by breast feeding are not severely affected by this virus.

**Slide 40**

CMV is the cause of the most common congenital viral infection in the USA. There are an astonishing 40,000 annual cases (1% of our annual births), but fortunately most of these infections are inconsequential. Annually about 3000 of these infected babies, however, will have symptoms at birth such as prematurity, jaundice, microcephaly, and rash; their prognosis is poor. About 8000 more will appear normal at birth but will develop deafness and mild degrees of mental retardation as they grow older. For obvious reasons there is great interest in developing a vaccine against this infection, but so far none has been successful.

A good deal is known about the natural history of this fetal infection. The risk to the infant is highest when the mother is infected in the first trimester (first 13 weeks of pregnancy). A primary infection in the mother poses the greatest risk. Reinfection with CMV is common, but fortunately it is rare for a pregnant woman who is reinfected with CMV during pregnancy to have a significantly affected infant.
In terms of prognosis, it is useful to be able to distinguish between congenital infection and perinatal infection, which is acquired at birth and is inconsequential. The only way to do this is to culture urine from the baby for CMV in the first 3 weeks of life, and this is usually not done. In practical terms, it is often very difficult clinically to distinguish between congenital and perinatal infection. There are many more infants with perinatal infection than with congenital infection. There is no good therapy for infants with significant congenital CMV.

**Slide 41**

CMV infections in immunocompromised patients are frequent and may be severe. They may be primary or recurrent, due to reactivation from latency. There are many strains of CMV, although there is only 1 antigenic type, and therefore reinfections are common.

Symptoms and signs in these patients include fever, pneumonia, retinitis, colitis, lymphadenopathy, rash, encephalitis, and neutropenia.

Diagnosis can be especially difficult. While the virus can easily be demonstrated in body tissues or fluids, by culture or PCR, proper interpretation of the results is the problem, because it is necessary to distinguish actual disease from persistent low grade viral multiplication, which may be innocuous. Asymptomatic CMV infections may occur even in immunocompromised patients. Fortunately with the use of protease inhibitors to treat HIV-infected patients, CMV infections have become less severe than they were in the past, because the patients are less immunocompromised.

**Slide 42**

There are a number of diagnostic approaches to CMV infections. Tissues can be examined for the presence of inclusion bodies that suggest CMV on both H&E and Pap staining. CMV can be cultured from secretions or tissues; this is expensive and takes time. The greater the viral load or titer, the faster the recovery of virus from a culture. CMV can also be demonstrated by immunofluorescence; combination of culture and immunofluorescence speeds up the diagnostic process. For diagnosis of congenital CMV infection, culture of the infant’s urine in the first 3 weeks of life is the best approach. Urine culture after that time cannot distinguish between congenital and perinatal infection with CMV.

Measuring antibody titers for CMV on acute and convalescent sera are of limited value because of the time involved to obtain the convalescent serum sample. IgM titers for CMV have a high degree of false negative and false positive determinations.
Molecular techniques such as PCR and in situ hybridization are becoming more common and useful for diagnostic purposes.

Slide 43

CMV infections cannot be treated with ACV and require use of more toxic drugs such as ganciclovir, foscarnet, and cidofovir. These medications usually must be given intravenously and cause much more toxicity than ACV. Various toxicities include significant bone marrow suppression and metabolic irregularities. Because the drugs are all potentially toxic, accurate diagnosis is obviously very important. Most of the treatment of CMV disease is in immunocompromised patients with very significant illnesses.

Drugs that are used to treat CMV infections all act by inhibiting viral DNA polymerase. Ganciclovir is related to ACV, but more toxic; its main toxicity is bone marrow suppression. This is so frequent and severe, that treatment has to be discontinued in about 20% of patients who receive it. Still ganciclovir remains the drug of choice to treat significant CMV infections. Toxicity of foscarnet includes renal damage and metabolic abnormalities. Toxicity of cidofovir includes renal damage and increases in uric acid. Because of its toxicity it is rarely used.

Diagnosis and treatment of CMV have become intertwined, as current thinking is to try to identify significant infections very early in their course and to treat preemptively, which yields the best outcomes.

Slide 44

A great deal is known about transmission of CMV. Transmission is associated with close personal contact. Transmission can be sexual, and various secretions such as saliva, tears, and urine are known to contain infectious CMV. Infectious virus can persist on surfaces for periods long enough to transmit virus to others. Children in day care are well-known transmitters of CMV to others via their infected saliva and urine. For other children this transmission is inconsequential, but when they transmit CMV to susceptible adults who may be pregnant, there can be serious consequences.

CMV is rarely airborne as it is highly cell-associated and does not produce skin lesions. Probably because the virus is not airborne, transmission from infected patients to hospital workers is rare.

Intrauterine transmission of CMV occurs, as well as infection through breast milk. CMV may also be transmitted through blood transfusion and organ transplantation.

Slide 45
Understanding how CMV spreads has enabled us to develop practices that interfere with transmission and help to control the virus. Proper handwashing is a major means of impeding transmission of CMV, for example after examining patients and also after diapering infants.

Use of condoms and/or abstinence can also prevent sexual CMV transmission.

For high risk patients several approaches to control are used. Transplant patients are tested before transplantation and the organ donor is also tested. The greatest risk is when the person transplanted is CMV antibody negative and the donor is CMV antibody positive. Lesser risks are present when the recipient is known to be CMV positive. However, all transplant patients are at substantial risk from CMV. It is also useful to test blood for CMV positivity prior to transfusion. Optimally, CMV seronegative blood is safest for transfusion of immunocompromised patients. CMV positive blood may be irradiated and/or filtered to remove infectious CMV.

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Infections with Epstein Barr Virus (EBV) may be very complex. The virus can cause lytic, latent, and immortalizing infections. This large gamma herpesvirus was discovered about 35 years ago in tissues from patients with Burkitt’s Lymphoma. It was recognized to cause infectious mononucleosis (IM) when a laboratory research worker was inadvertently infected with the virus and developed IM, after which time Koch’s postulates were fulfilled.

The major gp of EBV is designated gp 350, which binds to CD21 (the C3d complement receptor) on B lymphocytes. Interestingly, therefore, patients with x-linked agammaglobulinemia cannot be infected with EBV.

Seropositive asymptomatic individuals often shed virus in their saliva and can infect others. This is one of the few instances of lytic infection with EBV in the body in immunocompetent persons.

Like CMV, EBV is capable of immune evasion. It carries genes that mimic IL10, decrease interferon (IF) production, and inhibit apoptosis.

There is no good specific treatment for EBV infections. Fortunately it is needed rarely, but is especially important for some immunocompromised patients with severe infections or tumors caused by EBV. Current experimental approaches in these situations include decreasing immunosuppressive therapy if possible, giving monoclonal antibodies (rituximab), and infusion of immune lymphocytes.

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The main illness that is associated with EBV is IM. In addition, however, this tumor virus causes the following problems: nasopharyngeal carcinoma, lymphomas (including Burkitt’s), oral hairy leukoplakia in patients with AIDS, and the x-linked lymphoproliferative syndrome (in males only).
In IM, B lymphocytes are latently infected with EBV. These cells are then stimulated to form a variety of antibodies termed “heterophile”. The normal immune response is mediated by T lymphocytes, which form the “atypical lymphocytes” classically seen on blood smears in this disease.

Latent EBV persists in memory B cells.

EBV is not related to a puzzling clinical illness called chronic fatigue syndrome. Whether there is a chronic EBV syndrome that results in long-term symptoms is open to question. Very rarely IM has been observed to progress to lymphoma within a short time.

There is no specific therapy for IM, which is usually self-limited. Steroid therapy has been employed to treat certain complications such as airway obstruction due to enlarged lymph nodes, hemolytic anemia, and very rare severe cardiac and neurologic complications.

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IM is clinically a well-defined illness that usually occurs in young adults. Symptoms and signs include fever, lymphadenopathy, exudative pharyngitis, hepatosplenomegaly, and fatigue. Patients who are inadvertently treated with ampicillin can develop a characteristic immune-mediated maculopapular rash.

A tentative diagnosis can be made by performing a monospot test to demonstrate heterophile antibodies, which are formed during acute IM. Definitive diagnosis is made by demonstration of specific antibodies. Antibodies to the viral capsid antigen (VCA) develop early in IM and persist for life. Antibodies to the EBV nuclear antigen (EBNA) develop late and also persist for life. Therefore blood that is positive for anti VCA and negative for anti-EBNA is diagnostic of acute mononucleosis.

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The beta human herpesviruses 6 and 7 were first recognized about 15 years ago. HHV-6 is is best known for causing the acute disease roseola in infants. These babies present with irritability and high fevers that may cause seizures; as their fever falls, the typical maculopapular rash occurs. For most babies, this is a self-limited illness. However, high fevers in small babies are always a cause for concern because they may be indicative of many potentially serious causes of infection. There is a great deal of speculation as to whether or not there may be long-term sequelae of HHV-6 following roseola, because it appears to become latent in the CNS. HHV-6 also causes fever in immunocompromised persons and a rare IM-like syndrome.

HHV-7 is also a cause of fevers in immunocompromised patients.
These infections usually do not require specific treatment as most are self-limited. They may present difficult diagnostic problems in high-risk patients.

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HHV-8 is closely related to EBV and is a *gamma* herpesvirus. It is a large virus that not only encodes its own genes but has pirated a number of human genes to add to its own repertoire. These include genes encoding IL-6, Bcl-2 (antiapoptosis), and genes encoding chemokines.

Infections in children with this virus are rare, but include a non-specific rash illness.

This virus causes Kaposi’s sarcoma in HIV-infected patients and in the elderly. It also causes primary-effusion lymphoma, and Castleman’s disease. There is no effective antiviral therapy.

In summary, the herpesviruses cause a number of significant human infections and some are also associated with tumors. Antiviral therapies are available against a number of these viruses and have led to significant improvements in their morbidity and mortality. Nevertheless herpesviruses still cause a number of serious illnesses, particularly in the fetus and newborn infant and in immunocompromised patients. While there is currently a licensed vaccine available for only one of the herpesviruses (VZV), effective vaccines are projected soon to be developed for some of the others.