

VARICELLA-ZOSTER VIRUS EPSTEIN BARR VIRUS, AND HUMAN HERPESVIRUSES (HHV) 6, 7, AND 8

BACKGROUND

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. In general, 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 (or similar rash elsewhere on the body), which can be painful, annoying, and facilitates transmission of the virus 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, continuous low grade virus multiplication rarely can occur, often with some symptoms. This is called persistent infection and is a form of lytic replication.

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

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).

Betaherpesviruses 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.

Gammaherpesviruses 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.

Transcription of the 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 encode 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.

All of the herpesviruses are similar in appearance on electron microscopy. They are composed of a phospho-glycolipid envelope, a tegument, an icosahedral (20-sided) capsid, and a DNA core.

BASIC VIROLOGY OF VARICELLA-ZOSTER VIRUS (VZV)

Varicella-zoster virus (VZV), which causes chickenpox and zoster, serves 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 these viruses.

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 replication and also maintenance of latent infection, and is a lynchpin for synthesis of VZV.

The VZV genome can be diagrammed, and similar diagrams have been developed for the other human herpesviruses. The VZV genome, which is the smallest of the herpesviruses, is divided into unique long and short sequences, which contain internal and external repeats.

- U = unique
- L = long
- S = short
- T = terminal
- R = repeat

Three receptors for VZV have been identified: heparan sulfate proteoglycan, the mannose 6-phosphate receptor (MPR), and the insulin degrading enzyme (IDE).

Newly synthesized cellular proteins are sorted for exit from cells at the Golgi. MPRs are normally present in human cells at the trans-Golgi network and cell surface and play a role in the sorting of some cellular proteins into endosomes or lysosomes. VZV follows this pathway during egress because its envelope contains mannose 6-phosphate.

Viral Replication

DNA replicates in the nucleus and nucleocapsids are formed there; these are extruded into the perinuclear space. A primary envelope surrounds the capsid, but this is not the final envelope of VZV.

Nucleocapsids with an early envelope progress to the cytosol, where they are extruded as their envelope is fused with rough endoplasmic reticulum (RER), releasing the naked nucleocapsid into the cytosol of the infected cell.

Glycoproteins are formed in the RER and transported independently to the Golgi. Tegument proteins are formed in the cytosol and are also transported independently to the Golgi. In the trans Golgi network (TGN), due to processes of protein signaling, nucleocapsids, tegument, and glycoproteins all come together to form the enveloped virion.

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 one cell to another cell. Infectious VZV does not emerge from the cell. Therefore in host defense, VZV must be attacked with a cell-mediated response which can kill cells harboring the virus. The antibody response is not helpful because antibodies can only neutralize extracellular virus. Antibodies do not penetrate infected cells so cannot reach VZV, which is thus an intracellular pathogen.

There is one place in the human body where infectious, extracellular VZV is formed. This is in the skin, in the superficial epidermis. There are no MPRs in this part of the epidermis, so that VZV does not enter endosomes and it is extruded by the default pathway. These cell-free infectious virions are highly infectious, can be aerosolized, and play a major role in spread of VZV to others. They are also the form of the virus that can establish latent infection.

CLINICAL INFORMATION ON VARICELLA-ZOSTER VIRUS (VZV)

The primary infection with VZV is **varicella(chickenpox)**, a highly contagious disease spread by the airborne route. This illness has an incubation period of 10-21 days, with a viremic phase that precedes development of symptoms. Major symptoms are an itchy rash and fever, with associated malaise, headache, loss of appetite, etc.). Most patients are only mildly ill, but have skin vesicles are full of infectious VZV. This is the only time that enveloped virions are known to be produced in the body; otherwise cell to cell spread through the body is the rule. These cell free virions can be aerosolized, and this infection spreads by the airborne route from the skin of an infected person to the respiratory tract of a susceptible individual. Because it is usually not possible accurately to predict in advance who will develop severe varicella, the varicella vaccine was developed. Complications of varicella, include bacterial superinfection, encephalitis, pneumonia, congenital syndrome, and on occasion death.

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 due to cell to cell spread. 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 with an antiviral drug). This long incubation period is probably one reason why the varicella vaccine has been so effective in controlling varicella.

A number of complications of varicella are seen in babies.

One is the congenital varicella syndrome. This occurs when a baby's mother has varicella during pregnancy and infects her fetus. The intrauterine immune response of the fetus to the virus is immature, resulting in poorly checked viral replication. This is thought to lead to overwhelming latent infection and a high degree of fetal tissue damage. Many organ systems are affected, and these babies often die in infancy or early childhood. Fortunately this is a rare outcome of maternal infection (2%). It is likely that the problem will become even more rare because today most people are immunized against varicella.

Babies whose mothers have varicella just before delivery are at high risk to develop overwhelming varicella, due to immaturity of the immune system.

One of the youngest children on record to develop zoster is a 3 month old baby with classic symptoms, including a characteristic 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 zoster.

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 a product called VariZIG, which has very high titers of antibody to VZV. VariZIG must be given as soon as possible after exposure in order to work. It is only useful when given to a varicella susceptible. It will prevent zoster. People given VariZIG 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 an antiviral medication as soon as possible.

Immunocompromised patients who have had varicella are at increased risk to develop zoster, and they should receive antiviral therapy should they develop this illness.. 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.

Secondary VZV infection is zoster.

Zoster is due to reactivation of latent VZV; the latent virus was acquired during primary infection (chickenpox). This has been proven because VZV DNA, RNA, and proteins have been identified at autopsy in people who had chickenpox but 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 due to aging or disease/treatment for disease. Zoster is characterized by a unilateral painful or itchy rash in a dermatomal distribution. It may be accompanied by systemic symptoms such as fever, headache, and malaise. It is especially common in elderly and immunocompromised persons. During a lifetime, about 25% of persons who have had natural varicella will develop zoster. In the prevaccine era, virtually 100% of the US population developed chickenpox. The most serious complication of zoster is post-herpetic neuralgia (PHN), a neuropathic pain syndrome that may be excruciatingly painful and for which there is no good treatment. Recently a vaccine to prevent zoster by boosting cellular immunity to VZV has been developed and is recommended for healthy persons over 60 years old. It prevents about 50% of zoster cases.

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

The pathogenesis of latent VZV infection and zoster is as follows. During varicella, enveloped cell-free virions are present on skin and invade the sensory neurons without multiplying (which would kill the neuron). Latent VZV infection occurs only in neurons. By unknown means, the virus becomes latent. In latency, it is able to express at least 6 of its 71 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. All the viral genes are expressed in the lytic infection that results after reactivation.

In latent VZV infection 6 regulatory proteins (from IE and E genes) are expressed. In lytic infection, these proteins are nuclear, but in latent infection they are cytoplasmic. This observation suggests that the cascade of gene expression is blocked because the regulatory proteins are excluded from the nucleus in VZV latency

Live attenuated varicella vaccine

VZV is currently the only human 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 13 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.

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% usually 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.

Diagnosis of VZV infection

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

Diseases that present with vesicles can be diagnosed rapidly by performing immunofluorescence on a smear of the vesicular fluid.

Classic methods for diagnosis of VZV include: culture, direct immunofluorescence (DFA) and PCR on smears from a skin rash. Positive cultures for VZV often take 5-7 days to develop.

A rise in antibody titer is also diagnostic of infection. This testing 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.

Antiviral therapy

Effective antiviral therapy has changed the prognosis of herpesvirus infections, particularly in immunocompromised patients, in the past 25 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.

HSV 1 and 2, and VZV are both sensitive to ACV, although VZV is less sensitive than HSV. Patients with VZV infections have to be 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.

Epstein Barr Virus (EBV)

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.

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 therapy for EBV infections. Fortunately treatment is needed rarely, but it 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.

The main illness that is associated with EBV is infectious mononucleosis (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 thought to be 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.

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.

Human Herpesviruses 6, 7, and 8.

The *beta* human herpesviruses 6 and 7 were first recognized about 20 years ago. HHV-6 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.

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.

Summary

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.