Common Features of Herpesviruses

• Morphology
• Basic mode of replication
• Primary infection followed by latency
• Ubiquitous

• Ability to cause recurrent infections (reactivation of latent virus), reinfections (with a new virus), persistent infections (chronic low grade virus multiplication) immortalizing infections (EBV only)

8 Human Herpesviruses, 3 categories

• Alpha: short reproductive cycle, variable host range, latent in sensory neurons
  – Herpes simplex virus (HSV 1, 2)
  – Varicella-zoster virus (VZV)
• Beta: long reproductive cycle, narrow host range, latent in lymphoid cells & others (salivary glands, kidney)
  – Cytomegalovirus (CMV)
  – HHV6, HHV 7
• Gamma: narrow host range; latent in lymphoid cells, associated with tumors
  – Epstein Barr Virus (EBV)
  – Kaposi Sarcoma Virus (KSH, HHV8)
Human Herpesviruses

• Replication (lytic infection) occurs in a cascade
  – Latency occurs when the cascade is interrupted
• Transcription of viral genome and protein synthesis (cascade of gene expression), essential and luxury
  – 1. immediate early (IE): regulation of gene expression, DNA binding
  – 2. early (E): more transcription factors, enzymes, DNA polymerase
  – 3. late (L): structural proteins
• Encode targets for antiviral therapy
  – TK, DNA polymerase

Human Herpesvirus (VZV)
phospholipid envelope, tegument, icosahedral capsid, DNA core
VZV is a typical herpesvirus

- VZV has a phospholipid envelope. Contains gps, eg: gB, gC, gE, gH,gI, gK, gL
- Tegument contains immediate early and early proteins, eg: ORFps 4, 10, 21, 62, 63
- Nucleocapsid contains ORFp40
- DNA core

Varicella-zoster virus

- The smallest of the herpesviruses
  - 125,000 base pairs
  - 70 Open reading frames (ORFs)
- Receptors: heparan sulfate, mannose-6 phosphate receptor
MPRs sort lysosomal enzymes and target them to endosomes

Proteins mixed → Sorting → MPR-mediated diversion (endosomes/lysosomes)

Constitutive secretion; flow to surface

Signal-mediated diversion (secretory vesicles)

Steps in the assembly and intracellular transport of VZV
VZV receives its final envelope in the TGN

- Cytosolic nucleocapsids and tegument are wrapped by TGN cisternae.
- Concave surface = viral envelope.
- Convex surface = transport vesicle.

VZV Receives Its Final Envelope in the TGN
Herpes simplex virus (HSV) Infection (alpha)

• Cellular receptors are heparan sulfate, HVEM, Nectin-1, 2
  – Members of the immunoglobulin protein and TNF families
• Glycoproteins B, D, H, L promote attachment to cells and viral fusion with cell membrane
  – Viral proteins are released promoting gene transcription, cytotoxicity
• Lytic infection results in cell death
  – Virions are released or spread from cell to cell
• Latent infection occurs in sensory neurons
  – Latency associated transcripts (LATS)
  – Minimal transcription of DNA, no translation

HSV Infections

• Classification: primary, non-primary, first episode, recurrent, reinfection
• HSV-1: above belt, HSV-2: below belt
• Reactivation: trauma, sunlight, stress
  – Despite antibodies
  – May be related to deficient gamma IF response
  – May recur in same area of skin (unlike VZV)
• Many/most infections are asymptomatic
  – asymptomatic shedding can transmit HSV to others
• Host factors: immunocompromised, newborn baby
Herpes simplex virus (HSV) Infections

- Mucocutaneous, neonatal, CNS
- **Type 1**: gingivostomatitis, whitlow, keratitis, encephalitis, eczema herpeticum
- **Type 2**: genital, meningitis, neonatal
  - Main serious clinical problems are in newborn, and immunocompromised hosts
- Healthy hosts may develop gingivostomatitis, encephalitis, acute and recurrent genital HSV
- Disease from viral- and immuno-pathology

Primary HSV-1 gingivostomatitis
Herpetic whitlow, HSV 1

HSV causes about 1000 cases of encephalitis annually in USA

- Most common form of focal encephalitis in USA
- Primary or recurrent HSV-1; skin lesions may be present (not helpful for diagnosis)
- Symptoms, signs: headache, fever, personality change, focal seizures, abnormal EEG, CT, MR
- Differential diagnosis: TB meningitis, arbovirus, enterovirus, flavivirus, mycoplasma, tumor, toxoplasmosis, aneurysm
- Diagnosis: CSF culture is usually negative, but PCR is often positive for HSV
- Treat (ACV) if suspect disease; prognosis better in children than adults; early therapy is best
Perinatal HSV is usually due to Type 2 virus

- 95% neonatal, 5% congenital
- Usually the mother is asymptomatic
- Attack rate >10 times higher in maternal primary infection than recurrence; attack rate about 50%
- Clues: skin vesicles in 70%, fever, seizures, pneumonia, DIC, conjunctivitis
- Diagnosis: immunofluorescence, culture, PCR
- Treat all infants with this diagnosis, even if all they have are a few skin vesicles but seem otherwise well

Neonatal HSV-1
Neonatal HSV-2

Neonatal HSV Infection, 1600 cases annually

- Skin, eye, mucous membrane (40%)
  - Skin vesicles
  - Good prognosis with early treatment
  - Untreated 75% develop disseminated infection
- CNS Infection (35%)
  - Fever, lethargy, seizures, abnormal CSF
  - 50% mortality; major sequelae if survive
- Disseminated disease (25%)
  - Hepatosplenomegaly, jaundice, hepatitis, pneumonia
  - 2/3 develop skin vesicles
  - 70% mortality
Neonatal HSV

• Diagnosis: immunofluorescence, culture, PCR
  – Antibody titers are not useful

• Treat all newborn infants with possible HSV
  – Begin therapy while awaiting diagnostic results
  – Specific treatment (ACV) is very well tolerated

• Recurrent skin vesicles are associated with a poorer prognosis
  – may re-treat with ACV
  – May give 6 weeks of oral ACV

Natural History of VZV

• **Primary infection: varicella**
  – Highly contagious (airborne)
  – Complications: bacterial superinfection, encephalitis, pneumonia, congenital syndrome

• **Secondary infection: zoster**

• Zoster is due to reactivation of latent VZV
  – DNA, RNA, proteins in ganglia at autopsy
  – Zoster in a few vaccinees caused by Oka vaccine
  – From low cell-mediated immunity (CMI) to VZV

• No asymptomatic shedding of VZV as with HSV
Varicella is a generalized illness. Infectious virions are produced in the skin vesicles.

Zoster is initially localized.

- Limited to 1-3 dermatomes.
- May disseminate in immunocompromised hosts.
In the body VZV spreads from cell-to-cell

- In varicella, VZV is transported from the respiratory mucosa to the blood (viremia) in T cells, where virus is not accessible to antibodies.
  - Because cell-to-cell spread is slow, the incubation period of varicella is long (2 weeks).
  - Slow spread prevents host from being overwhelmed before the immune response develops
- T helper (TH1) and cytotoxic T cells are required for host control of virus

VZV spreads in two ways
Congenital varicella syndrome

Fatal neonatal varicella
Zoster in a 3 month old

VZV In the Immunocompromised

- Varicella is likely to be severe
  - Prevent or modify with pre-formed antibodies just after exposure
  - Virus spreads from cell-cell in body
    • requires CMI (cellular immunity) for host defense
  - Treat most immunocompromised patients immediately with acyclovir
- The frequency of zoster is increased
  - Probably related to low CMI response
  - Likely to suffer post-herpetic neuralgia (PHN) (also elderly)
Latent Infection with VZV

- Latent infection in dorsal root ganglia (DRG)
- 6 of 68 genes (also RNA and proteins) expressed during latency
- Proteins of regulatory genes are expressed in cell cytoplasm, not nucleus
- Suggests regulatory proteins are blocked from normal action, leading to inhibition of cascade of gene expression preventing lytic infection from occurring (latency)
- Latency is established when cell-free VZV in skin vesicles invades neurons
Varicella Vaccine

Only herpesvirus for which there is a vaccine
Live, attenuated, infectious virus (Oka strain)
Licensed for routine use in healthy susceptible individuals in US, in 1995
Recently there has been a marked decrease in varicella, in all age groups
  – Indicates herd immunity
• Contraindications: pregnancy, immunocompromised, allergy to vaccine components

Varicella Vaccine

Major complaint afterwards: mild rash in 5%
  • 1 month after vaccination; transmission to others is rare
  • This vaccine is extremely safe

85% completely protected; 15% partial immunity

There is little evidence for waning immunity

Subsequent zoster is rare
The rash of VZV is vesicular.

- Vesicular fluid is highly infectious.
  - Well-formed virions are suspended in it.
**Indirect immunofluorescence**

To diagnose VZV, HSV

**Laboratory Methods for Diagnosis**

- **Culture (difficult), DFA, PCR, cytology on skin rash (Tzanck)**
  - Can distinguish the Oka virus from wild type virus (PCR)

- **Antibody titers, IgG (ELISA)**
  - Acute serum, early in illness
  - Convalescent serum, 10-14 days after onset

- **Antibody titers, IgM**
  - False positives and false negatives can be a problem
Acyclovir (ACV) is useful to treat HSV, VZV

- Antiviral activity only in infected cells (TK)
- Sensitivity: HSV1, >HSV2, >VZV (EBV, CMV)
- Toxicity is unusual: gastrointestinal, neurologic (headache, seizures, delirium); anemia, thrombocytopenia, bone marrow suppression
- Resistance is a concern, especially in HIV-infected patients
- Newer drugs: famciclovir, valacyclovir
  - Administered orally and less frequently than ACV because better gastrointestinal absorption

Cytomegalovirus (CMV)

- Largest of the herpesviruses (mRNA too)
  - 208 ORFs; gB, gH
  - immune evasion
    - Down regulation of MHC class I expression to reduce effectiveness of cytotoxic T cells
- Host defense: cellular not humoral immunity
- Latency in bone marrow precursors of monocytic peripheral blood cells
  - Differentiation of monocytes into macrophages due to antigenic stimulation reactivates CMV
    - Adverse effects on transplantation
Cytomegalovirus (CMV)

- In healthy adult hosts infection is usually subclinical
  - Mononucleosis-like syndrome occurs but is rare
- Severe, opportunistic infections in immunocompromised hosts
  - AIDS patients, after transplantation
- Fetal (congenital) infections: can be severe
- Perinatal infections: of little consequence
  - At birth (maternal secretions), from breast milk

Congenital CMV Infection

- Most common congenital viral infection in US
  - 40,000 annual cases (1% of all infants)
    - 3,000 symptomatic at birth (jaundice, petechiae, microcephaly, prematurity)
      - 8,000 with sequelae (deafness, retardation)
- Risk to the infant is highest in first trimester (13 weeks) maternal infection
  - primary maternal infection poses greatest risk
  - the fetus is not always protected when an “immune” mother is re-infected with a different strain of CMV
- Distinguish between congenital and perinatal
  - In congenital infection urine is culture + for CMV in first 3 weeks of life
CMV Infections in the Immunocompromised (including AIDS)

- Frequent
- May be primary or recurrent (reactivation from latency)
- Can have reinfection with a new strain
- Symptoms/signs: fever, pneumonia, retinitis, colitis, lymphadenopathy, rash, encephalitis, neutropenia, etc.
- Diagnosis is difficult; must distinguish between true infection/disease and persistent virus
  - Asymptomatic infections occur

Diagnosis of CMV

- Histology: has limitations (not specific)
  - Basophilic inclusion bodies
  - H&E, Pap staining
- Cell culture
  - Cytopathic effect, immunofluorescence
- Serology: acute and convalescent antibody titers are of limited value
  - False positive and false negative IgM titers
- In situ hybridization
- PCR
Treatment of CMV

- **Ganciclovir**
  - Phosphorylation by viral enzymes causes inhibition of viral DNA polymerase (related to acyclovir); toxicity: bone marrow suppression
- **Foscarnet**
  - Inhibits viral DNA polymerase; renal, metabolic toxicity
- **Cidofovir**
  - Inhibits viral DNA polymerase
    - Very toxic (renal, uric acid increase)

**Pre-emptive approach**
- Identify infection before the illness
- Treatment used mostly for immunocompromised patients

Transmission of CMV

- **Close personal contact**
  - Sexual, day care (saliva, tears, urine)
  - Virus is not usually airborne
    - Cell-associated virus, no skin lesions
    - Spread from secretions, on hands
- **Intrauterine/birth/breast milk**
- **Transfusion**
- **Transplantation**
Control of CMV

• Hand washing (eg, after diapering)
• Condoms, abstinence
• Beware of blood
  – Use seronegative, irradiated, filtered blood for high risk patients
• Testing for CMV in transplantation (donor, recipient)
• Vaccine still not available

Epstein-Barr Infections (gamma)

• Major glycoprotein is gp 350 which binds to CD21 on B cells (C3d complement receptor)
  – Patients with x-linked agammaglobulinemia can’t be infected
• Seropositive persons shed virus in saliva (lytic)
• Virus utilizes immune evasion
  – Genes that mimic interleukin (IL) 10 and decrease interferon (IF) response, inhibit apoptosis
• Experimental therapy for immunocompromised patients with severe infections/tumors (lymphoproliferative disease)
  – Decrease immunosuppressive therapy if possible
  – Monoclonal antibodies (rituximab)
  – Infusion of leukocytes
Epstein-Barr Infections (EBV)

- **Infectious mononucleosis**, nasopharyngeal carcinoma, lymphomas (including Burkitt’s), oral hairy leukoplakia (lytic infection), X-linked proliferative disease (males only)
- B cells are latently infected in mononucleosis; T cells (atypical lymphocytes) are the host response
- Latency develops in memory B cells
- EBV is not related to chronic fatigue syndrome, but rarely severe chronic illness follows mononucleosis
- In mononucleosis, give steroids if airway obstruction, hemolytic anemia, severe cardiac, neurologic disease (no specific antiviral therapy)

Diagnosis of Mononucleosis

- Usually occurs in young adults
- **Symptoms, signs**: fever, adenopathy, exudative pharyngitis, rash (ampicillin), hepatosplenomegaly, fatigue
- **Positive heterophile antibody** (monospot)
- **EBV specific antibodies**
  - Anti VCA (develops **early**, persists)
  - Anti EBNA (develops **late**, persists)
  - Positive aby VCA, neg aby EBNA = acute mononucleosis
Herpesviruses 6, 7

• Herpesvirus 6 (beta, like CMV)
  – Roseola in infants (rash, fever, seizures)
    • outcome of latency in CNS not understood
  – Fevers in immunosuppressed
  – Rare mononucleosis syndrome in adults
• Herpesvirus 7 (beta, like CMV)
  – Fevers in immunocompromised (HIV)
• Diagnosis, treatment are not fully developed
  – Most infections are self-limited

Herpesvirus 8 (KHSV)

• Closely related to EBV
• Encodes for human proteins (piracy)
  – IL-6, Bcl-2 (anti-apoptosis), chemokines
Infections are rare in children
  – Can cause non-specific fever and rash illness
• Causes Kaposi’s Sarcoma
  – Elderly
  – HIV-infected
• Causes primary-effusion lymphoma
• Castleman’s disease (lymphoma-like)
Summary: Herpesvirus Infections

- Particularly affect newborns, elderly, immunocompromised
  - Congenital (CMV, VZV) vs neonatal (HSV, VZV); primary maternal infections high risk
- Primary, latent, recurrent, reinfections
- Best diagnostic tool: PCR
- Antiviral therapy: HSV, VZV, CMV
- EBV and HHV8 cause tumors
- Vaccine now available against to prevent chickenpox (varicella)