Persistent Infections

Lecture 16
Virology W3310/4310
Spring 2012
“Breaking Up Is Hard To Do”
Neil Sedaka 1962
Acute vs. Persistent Infections

- Acute - a natural infection that usually is rapid and self limiting

- Persistent - a natural infection that can be long term
  - slow
  - abortive
    - latent
  - transforming
Patterns of Infection

Recrudescence

- Acute infection
  - Rhinovirus
  - Rotavirus
  - Influenza virus

- Persistent infection, smoldering
  - Lymphocytic choriomeningitis virus

- Persistent infection, latent
  - Herpes simplex virus

- Persistent infection, slow
  - Measles virus SSPE
  - Human immunodeficiency virus
  - Human T-lymphotropic virus
Antigenic Variation

- Rhino, Influenza & HIV
  - selective pressure can lead to shedding of virions that are resistant to clearing
    - antigenic drift
    - selection
Persistent Infections

- Occur when primary infection is not cleared by the adaptive immune response
  - virus, genomes and/or proteins continue to be produced for years

- Chronic vs. Latent
  - chronic infections are eventually cleared
  - latent infections persist for a lifetime
General Properties of Latent Infections

• Gene products promoting replication are
  - not made
  - found in low concentrations
  - aberrantly localized

• Cells with latent genomes are masked from immune surveillance

• Viral genomes persist intact to reactivate and spread to a new host
  - except for measles and SSPE
Examples of Latent Infections

- Epstein Barr Virus (EBV)
  - novel transcription and replication pattern
  - no new virus
  - but genome replicates

- Adenoviruses
  - isolated from lymphoid tissue, adenoids and tonsils
  - cultured lymphocytes don’t support efficient virus replication
### Other Examples of Persistent Infections

<table>
<thead>
<tr>
<th>Virus</th>
<th>Site(s) of persistence</th>
<th>Consequence(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Adenoids, tonsils, lymphocytes</td>
<td>None known</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>B cells, nasopharyngeal epithelia</td>
<td>Lymphoma, carcinoma</td>
</tr>
<tr>
<td>Human cytomegalovirus</td>
<td>Kidneys, salivary gland, lymphocytes, macrophages, stem cells, stromal cells</td>
<td>Pneumonia, retinitis</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Liver, lymphocytes</td>
<td>Cirrhosis, hepatocellular carcinoma</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>Liver</td>
<td>Cirrhosis, hepatocellular carcinoma</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>CD4+ T cells, macrophages, microglia</td>
<td>AIDS</td>
</tr>
<tr>
<td>Herpes simplex virus types 1 and 2</td>
<td>Sensory and autonomic ganglia</td>
<td>Cold sore, genital herpes</td>
</tr>
<tr>
<td>Human T-lymphotropic virus types 1 and 2</td>
<td>T cells</td>
<td>Leukemia, brain infections</td>
</tr>
<tr>
<td>Papillomavirus</td>
<td>Skin, epithelial cells</td>
<td>Papillomas, carcinomas</td>
</tr>
<tr>
<td>Polyomavirus BK</td>
<td>Kidneys</td>
<td>Hemorrhagic cystitis</td>
</tr>
<tr>
<td>Polyomavirus JC</td>
<td>Kidneys, central nervous system</td>
<td>Progressive multifocal leukoencephalopathy</td>
</tr>
<tr>
<td>Measles virus</td>
<td>Central nervous system</td>
<td>Subacute sclerosing panencephalitis, measles inclusion body encephalitis</td>
</tr>
<tr>
<td>Rubella virus</td>
<td>Central nervous system</td>
<td>Progressive rubella panencephalitis</td>
</tr>
<tr>
<td>Varicella-zoster virus</td>
<td>Sensory ganglia</td>
<td>Zoster (shingles), postherpetic neuralgia</td>
</tr>
</tbody>
</table>
How to Promote Persistence

• Failure of innate immune system to clear an acute infection

• Blocking apoptosis can lead to persistence
Host Contributions to Persistence

• Eyes and neurons are devoid of initiators and effectors of the immune system
  - a vigorous immune response would be detrimental to the host

• Persistent infection of these organs is therefore common
State of the Genome

• Nonreplicating genome in a nondividing cell
  - HSV and VZV in neurons

• Autonomous self replicating chromosome in a dividing cell
  - HPV, HCV, HBV and EBV, KSHV

• Integrated in host chromosome, replicates with host
  - Parvoviruses
  - HHV6
Sindbis Virus

• Injection into adult mouse brain results in persistent, noncytopathic infection

• Injection into neonatal mouse brain results in lethal infection

• Why? It’s all about the milieu
  - neonatal neurons lack proteins that block virus-induced apoptosis
BDV a Pestivirus

• Two strains of virus
  - cytopathic (C) and noncytopathic (NC)

• Following in utero infection NC establishes a lifelong infection of cattle
  - these animals have NO detectable antibody or T-cell response to virus antigens
  - host is tolerized

• When infected with C, IFN response is activated and virus is readily cleared
Measles a Paramyxovirus and SSPE

- No animal reservoir
  - highly contagious
  - $4 \times 10^7$ infections/yr
  - systemic immunosuppression
  - lifelong immunity
Infection Pattern

- Pleomorphic particles: 100–300 nm
- Hemagglutinin
- Fusion protein
- Phosphoprotein
- Lipid bilayer
- RNA (16 kb)
- M (matrix protein)
- N (nucleocapsid)

Infection process:
- Primary viremia
  - Draining lymph nodes
  - Spread to entire RES

Secondary viremia
- Spread to all body surfaces
- Epithelial necrosis
- Virus shedding

Giant cells in infected tissue
- Antibody

Days after infection:
- 0: Incubation
- 2: Prodromal
- 6: Rash
- 16: Recovery

Rare complications:
- Encephalitis
- Subacute sclerosing panencephalitis
SSPE - Hypothesis

- Measles enters brain in infected lymphocytes
- Antibody blocks cell-cell fusion
  - removal of fusion protein from surface allows
    persistence of portions of virus
  - a slow infection, not persistent
- Low levels of envelope, no virions but
  nucleoprotein complexes spread from cell to cell
- SSPE develops after 6-8 years
Herpesvirus Latency Primer

• α HSV, VZV are neurotropic
  - default pathway lytic

• β CMV, HHV6 variable but prefer cells of lymphoid origin
  - default pathway lytic

• γ EBV, KSHV markedly lymphotropic
  - default pathway latency
Control of Latent Herpesvirus Genomes

- HSV - LAT transcripts derived from a single region of the chromosome accumulate

- VZV - small subset of aberrantly localized proteins may accumulate

- EBV - virus proteins and small viral RNAs are synthesized
  - required to maintain the latent state
  - modulate host response

- HCMV & KSHV - micro RNAs are thought to play a role in establishment of latency
Acquisition of CytoMegalovirus
HCMV

• Infects epithelial and other cell types
• Most infections are subclinical
• Cell-mediated immunity required for resolution of infection
• Establishes latency in bone marrow progenitors and macrophages
• Repression of CMI leads to recurrence
HCMV Infections

• Infection *in utero* can be devastating

• Early childhood, less so
  - virus persists
  - found in salivary and mammary glands and semen

• Reactivation can be with dire consequences
  - blood transfusion
  - organ donations

• miRNAs expressed by CMV *in vitro* and *in vivo*
  - are tissue specific
  - associated with a specific stage of viral infection
The First Rule of Latency

- Without reactivation there is no latency
- Without reactivation there is no advantage as the virus can no longer spread.
HSV Infections

- Population is >80% seropositive
- $\sim 2.5 \times 10^8$ have latent virus
- $4 \times 10^7$ will experience recurrence
  - some asymptomatic shedding
Both sensory and sympathetic ganglia can be infected
Postinfection Events in Neurons

- Nucleocapsid travels up the axon
  - VP16 is separated from nucleocapsid
- Limited productive infection
  - Local inflammation leads to resolution
- Genome is silenced and coated by nucleosomes
- Multiple copies of virus DNA
- Nuclear accumulation of LATs
What Do LATs Do?

- LAT− virus reactivates poorly
- 2 ORFs are contained in the LAT sequence but no known protein has been associated with them
- Encode MIRs that could inhibit expression of
  - ICP0, a potent transcriptional activator
  - γ34.5 a neurovirulence gene, it activates PPla
Why Neurons?

- Neurons don’t replicate or divide, genome is established and readily persists
- Insensitive to antivirals and immune surveillance
  - blood brain barrier
- But.......how do they survive the 1° infection?
- Why are there multiple copies of virus DNA?
Reactivation

- Only a small number of neurons in a ganglion reactivate
- Virions appear in mucosal tissue innervated by latently infected ganglia, blisters ensue
- What happens to surrounding neurons post reactivation?
- Many times reactivation is silent, virus is shed
- How is virus infection masked from host immune response?
Reactivation Triggers

- What flips the switch?
- Stress
- Glucocorticoids
- In a model system exogenous ICP0 can reactivate
- The VP16 conundrum
Chicken Pox vs. Shingles

Primary Viremia
- Infection via conjunctiva and upper respiratory tract
- Replication in primary lymph nodes

Secondary Viremia
- Replication in liver, spleen, and other organs

Day 0

Day 4–6

Day 14

Infection of skin and appearance of rash
- Sensory neurons
- Sensory ganglion
- Satellite cells

Reactivation

Infection of sensory ganglia and establishment of latent infection

To central nervous system
EBV a γ Herpesvirus

• 95% of adults are seropositive and carry the genome

• Virus resides in persistently infected non-proliferating memory B lymphocytes

• Causal agent of:
  - Hodgkins lymphoma
  - Infectious mononucleosis
  - Nasopharyngeal carcinoma
  - Burkitt’s lymphoma
EBV Lifecycles

Primary infection

Persistent infection

EBV

Saliva

Oropharynx

Epithelium

Dermis

EBV-infected B-cell clast

LMP-1

EBNA1

LMP-2

Latently infected, resting memory B cells

Cytotoxic T cell

Natural killer cell

Blood vessel

EBNAs

Reactivated EBV-infected B cell

LMP-2

LMP-1

LMP-2
Epigenetic Marking and EBV Replication

- DNA unmethylated
- Immediate early gene expression (Zta) - mode of action
- Subsequently methylated but Zta $t_{1/2}$ is short
Latently Infected B Cells & EBV

- Virus chromosome is a self-replicating episome
- Associates with nucleosomes
- Is methylated at CpG residues
- Expresses limited repertoire of virus genes
- Cells home to bone marrow and lymphoid organs
- Are not seen by CTLs or virus-specific antibody
- Virions produced in a very small fraction of cells
EBV Latency Programs

Progression of Naive B cell through germinal center to become Memory B Cell
EBV Latency Program
What Happens When B Cells Divide?

- Episomal virus genome has to replicate to be distributed to daughter cells
- EBV has two Origins for DNA replication
  - Ori Lyt is used for lytic replication
    - high copy #
  - Ori P is used for episomal replication in latently infected cells
    - low copy #
Cell-cycle Regulation of EBV DNA Replication During Latency

- Replication of episomal, nucleosome coated, virus genome is synchronized with the host
  - Why?

- oriP is normally quiescent
  - bound by host regulatory proteins (cdc6, cdt1)

- EBNA-1 interacts with host proteins to form a stable complex Origin Recognition Complex
Replication Licensing
EBV Latent Infection

- EBV replicates in synchrony with the cell
- Replication is licensed by formation of ORC
  - recruits other proteins (mcm)
  - release regulators, initiate DNA replication
- Late in S, geminin is produced and it sequesters Cdt1, geminin is subsequently degraded in G2, freeing Cdt to reassociate with ORC
- No second round of replication because during S and G2, mcm and Cdc6 are destroyed
HHV6 a β Herpesvirus

- Causal agent of a mild childhood disease
  - Exanthum subitum
  - 90% of population is seropositive

- Persistently infects the host for life
  - No circular episomal forms
  - Integrates into telomeres
  - Reactivates in the immunosuppressed

- Makes integration a plausible molecular strategy for viral latency
Human Papillomaviridae

• There are over 100 distinct types of HPVs
  - Genomes that vary by >10%

• Segregate in mucocutaneous and cutaneous types
  - high and low risk types
Papillomavirus DNA Replication

- Infect basal layer of differentiating epithelium
  - first replicate as episomes as cells divide
  - replication as theta forms “θ”

- Replicate virus genomes in terminally differentiated epithelial cells
  - interrupt program of terminal differentiation, express HPV E6 and E7
Papillomavirus DNA Replication

- Keratinized dead skin cells
- Epidermal cells
- Basal lamina

More differentiated skin cells

Precursor skin cells

Productive replication in differentiated cells

Limited amplification of episomal papillomaviral DNA

Maintenance Replication

Infection
Papillomavirus Replication

- E1 and E2 are homodimers
- E1 and E2 interact and bind cooperatively to ori
- E2 recruits E1
- Interaction elicits a bend in the DNA at the ori
- E2 dissociates - more E1 is recruited
Papillomavirus Persistence

- Intact virus genomes persist in basal cells of developing epithelium
  - genomes divide as episomes with host
  - infectious virus not present
Papillomavirus Persistence

- In developing cancers virus genome is integrated
  - replicates only when host cell divides
Human Polyomaviridae

- Six known members of the group
  - WUV, BKV, JCV, LPV, KIV and MCV

- Polyomaviruses can cause tumors in animal models
  - only MCV is associated with a human tumor
  - other human PVs appear to latently infect humans
Human Polyomaviridae

- Infection with JC or BK can lead to development of Progressive Multifocal Leukoencephalopathy (PML)
  - myelin is lost and not replaced by oligodendrocytes
  - nerves become damaged and over time stop working properly

- MS patients treated with Tysabri have a much higher than normal occurrence of PML
Human Polyomaviridae

• “Given the high seroprevalence of polyomaviruses in humans, it is not surprising that they are significant pathogens in immunosuppressed populations. An important question is why these viruses can peacefully co-exist in many humans without causing disease. Are human polyomaviruses simply passengers, or do they benefit us in some unknown way?”
  - VRR 2009 Blog
Merkle Cell Carcinoma
Polyomavirus
Clonal Integration

- Analysis of MCV DNA in MCC (a neuroectodermal tumor) shows it is integrated in a clonal pattern - therefore infection and integration preceded clonal expansion of the tumor cells

- MCV positive tumors have mutations in T - thus they are replication deficient

- integrated virus genomes are not excised - cells survive
Persistence

• Viruses preferentially target slowly dividing or nondividing cells to host their latent genomes

• They adopt a variety of survival strategies that coordinate replication of their genomes and expression from these genomes to allow them to persist

• In response to a variety of stimuli these latent genomes can on occasion reactivate