Persistent Infections

Lecture 16
Virology W3310/4310
Spring 2013
“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

- 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

Recrudescence
Antigenic Variation Can Lead to Persistence

- 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
## 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 includes 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
  - HIV in memory T cells

• 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
Measles a Paramyxovirus and SSPE

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

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

Infection:
- Primary viremia
- Secondary viremia
- Virus shedding
- Giant cells in infected tissue
- Draining lymph nodes
- Spread to entire RES
- Spread to all body surfaces
- Epithelial necrosis
- Antibody
- Disease
- Koplik’s spots

Days after infection:
- Incubation
- Prodromal
- Rash
- Recovery

Complications:
- Encephalitis
- Subacute sclerosing panencephalitis

Tuesday, March 26, 2013
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

- $\alpha$ HSV, VZV are neurotropic
  - default pathway lytic

- $\beta$ CMV, HHV6 variable but prefer cells of lymphoid origin
  - default pathway lytic

- $\gamma$ EBV, KSHV markedly lymphotropic
  - default pathway latent
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 lymphoid cells
• 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 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
- \( \sim 2.5 \times 10^8 \) in U.S. have latent virus
- \( 4 \times 10^7 \) will experience recurrence
  - some asymptomatic shedding
Both sensory and sympathetic ganglia can be infected by HSV.
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 that 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
Establishment, Maintenance & Reactivation

Primary herpes simplex virus infection

Viral activators

Cellular activators

Tegment proteins

Cellular repressors

Immediate-early genes expressed

Early genes expressed

Late genes expressed

Particle assembly

DNA packaging

Particle egress

Infectious virus produced

Inhibition of apoptosis

Modulate IFN and cytokine response

Inhibition of apoptosis

Cell-cell spread

Genome silencing

LAT expression

Inhibition of apoptosis

Latent infection established

Viral gene products?

Maintenance

Stimuli

Stress response

Viral and cellular activator expression (ICP0)

Latent infection

Reactivation

Productive infection

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Chicken Pox vs. Shingles

Infection via conjunctiva and upper respiratory tract

Replication in primary lymph nodes

Days 4–6

Primary Viremia

Replication in liver, spleen, and other organs

Secondary Viremia

Day 14

Infection of skin and appearance of rash

Reactivation

Infection of sensory ganglia and establishment of latent infection

Sensory neurons
Sensory ganglion
Satellite cells

To central nervous system

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**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

- EBV
- Resting B cell
- EBV-infected B-cell clast
- EBNAs
- Cytotoxic T cell
- Natural killer cell
- Blood vessel

Persistent infection

- EBV
- Lytic EBV-infected B cell
- EBNA-1
- EBNA
- Reactivated EBV-infected B cell

Location:
- Saliva
- Oropharynx
- Epithelium
- Dermis
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 only a small fraction of cells
Progression of EBV Infected Naive B cell through germinal center to become Memory B Cell

EBV Latency Programs

Migration to Peripheral Lymphoid Tissue

Activated B cell
(EBNAs-1,-2,-3a-c, -LP; & LMPs-1, 2)

Ag + T-cell help,
CD40, lymphokines

Naive resting B cell

Primary Lymphoid Follicle

Germinal Center
CD40, BAFF, LTα

Somatic Hypermutation
Affinity Maturation
Isotype Switching

Centroblasts
(EBNA-1 & LMPs-1, 2)

Centrocytes

Memory B cell
Long term latency reservoir (+/- EBNA-1)

Plasma cell
Virus reactivation
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?

• Ori P 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

- Only forms at G1-S boundary
- Produced late in S
- Only one round of DNA replication!
HHV6 a β Herpesvirus

• Causal agent of a mild childhood disease
  - Exanthum subitum, Sixth disease
  - 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 and vertical transmission
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

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

x 10^3
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 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
Merkel Cell Tumors
Merkle Cell Carcinoma

A

B Small T antigen

Hamster

Murine

MCV

African Green Monkey

KIV

WUV

BKV

SA12

JCV

SV40

MptV

Large T antigen

KIV

WUV

BKV

SA12

JCV

SV40

MptV

BFPyV

Finch

Crow

GHPV

Hamster

Murine

African Green Monkey

MCV

0.1

BPV

0.1

BPV
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