Herpesviruses: Latent and Lytic Infection

Vesicles, Cells and Disease

November 6, 2008
## Diversity within DNA viruses

<table>
<thead>
<tr>
<th>Classification</th>
<th>Example</th>
<th>Genome size</th>
<th># genes</th>
<th>envelope?</th>
<th>Unique features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyomavirus</td>
<td>SV40</td>
<td>5 kb. ds</td>
<td>7</td>
<td>NO</td>
<td>Transformation</td>
</tr>
<tr>
<td>Papillomavirus</td>
<td>HPV</td>
<td>8 kb. ds</td>
<td>10</td>
<td>NO</td>
<td>&gt;100 strains; Cervical cancer agent; Transformation following integration</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Ad2, Ad5</td>
<td>36 kb ds</td>
<td>14 tx. Units</td>
<td>NO</td>
<td>Leader RNAs; Common respiratory pathogen; Transformation; Gene therapy vector</td>
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<tr>
<td>Parvovirus</td>
<td>AAV</td>
<td>5 kb. ss</td>
<td>2</td>
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<td>Require helper virus; Gene therapy vector</td>
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<tr>
<td>Herpesvirus</td>
<td>HSV (HHV-1)</td>
<td>152 kb. ds</td>
<td>86</td>
<td>YES</td>
<td>Tegument</td>
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<tr>
<td></td>
<td>HCMV (HHV-5)</td>
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<td>222</td>
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<td>Latency</td>
</tr>
<tr>
<td></td>
<td>EBV (HHV-4)</td>
<td>172 kb. ds</td>
<td>82</td>
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<td>Transformation</td>
</tr>
<tr>
<td></td>
<td>KSHV (HHV-8)</td>
<td>170 kb. ds</td>
<td>86</td>
<td></td>
<td>Neural gene therapy vector</td>
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<tr>
<td>Poxvirus</td>
<td>Vaccinia</td>
<td>200 kb. ds</td>
<td>150</td>
<td>YES</td>
<td>Smallpox agent (Variola); Cytoplasmic replication (RNA pol, capping enz, poly(A) pol)</td>
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<tr>
<td>Hepadnavirus</td>
<td>HBV</td>
<td>3.2 kb ds*</td>
<td>8</td>
<td>YES</td>
<td>RNA repli. Intermediate; Hepatitis agent; liver cancer; 1st recomb. vaccine</td>
</tr>
</tbody>
</table>
Herpesviruses

- Extremely common, highly disseminated in nature
- >200 herpesviruses identified to date
- Every mammal is infected by at least one: Eight have been isolated from humans
- Spread through direct physical contact
- Alternating Lytic (Productive) infection and Latent (non-productive) infection
Virion Structure

Human Herpesviruses genomes:
150-235 kb. DNA, encode 80-225 proteins

Tegument: >20 proteins
## Classification: Three subfamilies -

### α herpesviruses:
- Human herpesvirus 1,2: **Herpes simplex virus 1, 2 (HSV-1, HSV-2)**
- Human herpesvirus 3: **Varicella-Zoster virus (VZV)**
  - a relatively rapid, cytocidal growth cycle
  - establish latent infections primarily in sensory ganglia

### β herpesviruses:
- Human herpesvirus 5: **Cytomegalovirus (CMV)**
- Human herpesvirus 6: **HHV-6**
- Human herpesvirus 7: **HHV-7**
  - the reproductive cycle is long and grow slowly in culture
  - establish latency in monocytes (CMV) or T cells (HHV-6, 7)

### γ herpesviruses:
- Human herpesvirus 4: **Epstein Barr virus (EBV)**
- Human herpesvirus 8: **Kaposi’s sarcoma associated herpesvirus (KSHV/HHV-8)**
  - establish latency in B cells
  - can transform cells; they are oncogenic herpesviruses
Although divergent in size and genomic architecture, herpesviruses share 7 conserved blocks of ancient genes.
Approximately 40 core genes are conserved among all herpesviruses

<table>
<thead>
<tr>
<th>Gene regulation</th>
<th>Virion</th>
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<tbody>
<tr>
<td>Transcriptional/post-transcriptional transactivator</td>
<td>Maturation</td>
</tr>
<tr>
<td></td>
<td>Alkaline exonuclease</td>
</tr>
<tr>
<td></td>
<td>Terminase/packaging</td>
</tr>
<tr>
<td></td>
<td>Capsid nuclear egress (2)</td>
</tr>
<tr>
<td></td>
<td>Genome cleavage/packaging (3)</td>
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</table>

<table>
<thead>
<tr>
<th>Nucleotide Metabolism</th>
<th>Capsid</th>
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<tbody>
<tr>
<td>Ribonucleotide reductase, large subunit</td>
<td>Major capsid protein (MCP)</td>
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<tr>
<td>Uracil DNA glycosylase</td>
<td>Minor capsid protein</td>
</tr>
<tr>
<td>dUTPase</td>
<td>Hexon tips</td>
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</table>

<table>
<thead>
<tr>
<th>DNA Replication</th>
<th>Tegument</th>
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</thead>
<tbody>
<tr>
<td>Helicase/primase (3 subunits)</td>
<td>Large tegument protein</td>
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<tr>
<td>DNA polymerase**</td>
<td>Protein kinase</td>
</tr>
<tr>
<td>Polymerase processivity factor</td>
<td>6 with unknown function</td>
</tr>
<tr>
<td>ssDNA binding</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other</th>
<th>Envelope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-to-cell fusion</td>
<td>Glycoproteins B (gB), gH, gL, gM, gN</td>
</tr>
</tbody>
</table>
Patterns of herpesviral replication must be considered at the cellular and organismal levels.
Reproduction and transmission determine the biological success of a virus.
Viral genes are expressed in a cascade fashion during replication of a DNA virus.
Productive gene expression cascade of herpesviruses

**Immediate Early (IE; \( \alpha \)) genes:**
- CHX resistant (no prior protein synthesis req).
- Transcriptional and post-transcriptional activation and de-repression.
- Inhibit IFN response
- Pro and anti-apoptotic
- Block antigen presentation
- Reduce host gene expression
- E3 ubiquitin ligase

**Delayed Early (DE; \( \beta \)) genes:** all require prior expression of combinations of IE transactivators
- Do not require prior viral DNA replication
- Can function as DE genes when present on a plasmid

**Late (L; \( \gamma \)) genes:**
- Expression enhanced by viral DNA synthesis
- Require IE transactivators + ssDNA binding protein (DE)
- \( \gamma 1 \)-expression does not require viral DNA synthesis
- \( \gamma 2 \)-expression strictly dependent on viral DNA synthesis
- Not understood--cis acting regulation
Small DNA viruses, but not herpesviruses, target central growth control proteins to replicate productively.

Small DNA Viruses (Papova and Adenoviruses)

- Viral early proteins (e.g. large T antigen, E1A)
- Rb-E2F
- Nucleotide biosynthesis genes OFF
- Deoxyribonucleosides, Ribonucleotides

Herpesviruses

- Viral IE proteins (e.g. HSV ICP4)
- Viral early biosynthesis genes OFF
- (e.g. HSV thymidine kinase)
- Deoxyribonucleosides
- Ribonucleotides
- dNTPs
Nuclear events--the goal is to replicate viral DNA
Viral coding capacity generally predicts autonomy vs. reliance on host functions

<table>
<thead>
<tr>
<th>Function</th>
<th>SV40</th>
<th>HSV-1</th>
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<tbody>
<tr>
<td>DNA pol</td>
<td>cellular DNA pol δ</td>
<td>UL30</td>
</tr>
<tr>
<td>Pol processivity</td>
<td>cellular PCNA</td>
<td>UL42</td>
</tr>
<tr>
<td>ssDNA binding protein</td>
<td>cellular RF-A</td>
<td>UL29</td>
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<tr>
<td>helicase/primase</td>
<td>Large T antigen</td>
<td>UL5,8,52</td>
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<tr>
<td>Origin binding protein</td>
<td>Large T antigen</td>
<td>UL9</td>
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<tr>
<td>RNAse H/5’-3’ exonuclease</td>
<td>cellular RNAse H, MF-1</td>
<td>UL30,42</td>
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<tr>
<td>Nucleoside phosphotransferase</td>
<td>cellular thymidine kinase</td>
<td>UL23</td>
</tr>
<tr>
<td>Ribonucleotide reductase</td>
<td>cellular</td>
<td>UL39,40</td>
</tr>
<tr>
<td>Deoxyuridine triphosphatase</td>
<td>cellular</td>
<td>UL50</td>
</tr>
<tr>
<td>Deoxyribonuclease</td>
<td>cellular</td>
<td>UL12</td>
</tr>
<tr>
<td>Uracil-DNA-glycosylase</td>
<td>cellular</td>
<td>UL2</td>
</tr>
<tr>
<td>Topoisomerase I, II</td>
<td>cellular</td>
<td></td>
</tr>
<tr>
<td>RF-C</td>
<td>cellular</td>
<td></td>
</tr>
<tr>
<td>Ligase I</td>
<td>cellular</td>
<td></td>
</tr>
</tbody>
</table>
**Lytic cycle (productive) herpesviral DNA replication**

**Requires:** Lytic origin of replication ("ori\_Lyt")

+ 7 viral proteins that participate directly at replication fork.

**Rolling circle replication**

Cleavage of genome concatemers into single units

Produces linear genomes that are packaged into capsids
DE gene products replicate viral DNA

1) Co-transfect mammalian cells with plasmids encoding 7 viral proteins:
   DNA polymerase
   Polymerase processivity protein
   ssDNA binding protein
   Origin binding protein
   3 helicase/primase proteins

2) Harvest viral DNA

3) Digest with Dpn I (digests transfected DNA ONLY) + specific REs

4) Southern blot for plasmid

Successful replication leads to viral budding and cell lysis
Herpesviruses establish latent, non-productive infections.
In herpesviral reactivation, the gene expression cascade is probably identical as in primary, productive infection.
Experimental Models for Latency and Reactivation of Human herpesviruses are few in number.

- Gamma-herpesviruses: Model: Herpes Simplex 1 and 2
  - Cellular level
  - Organismal level

Non-human herpesviruses provide animal models for others.
Herpesvirus Latency--Characteristics (cellular level)

1. Viral genomes persist as nuclear episomes:
   some herpesviruses express protein that tethers viral chromatin to host chromatin.
2. Viral DNA is nucleosomal.
3. Viral gene expression is extremely limited.
4. Viral DNA replicates along with host.
   Requires ori_{lat} + cellular replication machinery. Proceeds via a theta form.
   Yields circular progeny genomes.
5. Immune detection of the virus is reduced or eliminated.
6. Mature virions are not produced.
7. Establishment and maintenance of latency can quantitated separately. Establishment is not well-understood in herpesviruses.
8. Virus can be reactivated into productive cycle at a later time.
Herpesviral infection alternates between productive and non-productive replication for the life of the host (organismal level)

- **Acute infection**
  - Rhinovirus
  - Rotavirus
  - Influenza virus

- **Persistent infection**
  - Lymphocytic Choriomeningitis virus

- **Latent, reactivating infection**
  - Herpesviruses
Mechanisms that control productive vs. latent infection

Lack of expression of immediate early (IE) genes.
Expression is repressed by host cell factors
(transcription factors or repressive chromatin).

Expression is repressed by viral factors

Absence of host cell factors
  Lack of expression
  Lack of proper modifications
  Absence from nucleus

Inhibition of viral replication by the host immune response.

Evidence supports a combination of all of the above.

Balance between above mechanisms and those promoting productive infection probably determines outcome.
Specific players differ for different herpesviruses.
## Latent and Lytic Herpesviral replication is Cell and Disease-specific

<table>
<thead>
<tr>
<th>Classif.</th>
<th>Common name</th>
<th>LatentSite-Disease</th>
<th>Reactivation-Disease</th>
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</thead>
<tbody>
<tr>
<td>HHV-1</td>
<td>Herpes Simplex Virus-1</td>
<td>Neurons-none</td>
<td>Cutaneous Epithelium lesions-Face</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>or Genital</td>
</tr>
<tr>
<td>HHV-2</td>
<td>Herpes Simplex Virus-2</td>
<td>Neurons-none</td>
<td>Cutaneous Epithelium lesions-shingles, pain</td>
</tr>
<tr>
<td>HHV-3</td>
<td>Varicella/Zoster Virus</td>
<td>Neurons-none</td>
<td></td>
</tr>
<tr>
<td>HHV-4</td>
<td>NEXT SLIDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHV-5</td>
<td>Human cytomegalovirus</td>
<td>Monocytes/</td>
<td>Widespread Epithelium, and Endothelium-Fever, Retinitis, Cardiovascular disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Macrophages</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-none</td>
<td></td>
</tr>
<tr>
<td>HHV-6A</td>
<td></td>
<td>Monocytes/Macro-</td>
<td>Same-Bone Marrow Suppression, URI, AIDS dementia?</td>
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<tr>
<td>HHV-6B</td>
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<td>none</td>
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<tr>
<td></td>
<td></td>
<td>CD4+ T cells</td>
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</tr>
<tr>
<td></td>
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<td>-none</td>
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<tr>
<td>HHV-7</td>
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<td>CD4+ T cells</td>
<td>Salivary Epithelium</td>
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<td>HHV-8</td>
<td>NEXT SLIDE</td>
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</tbody>
</table>
Only Epstein-Barr Virus (EBV) and Kaposi’s sarcoma-associated Herpesvirus (KSHV) are conclusively associated with Human cancers.

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<thead>
<tr>
<th>Classif.</th>
<th>Common name</th>
<th>LatentSite-Disease</th>
<th>Reactivation-Disease</th>
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</thead>
<tbody>
<tr>
<td>HHV-8</td>
<td>Kaposi’s sarcoma-associated Herpesvirus</td>
<td>B cells- Primary Effusion Lymphoma</td>
<td>Lymphatic Endothelium-Kaposi’s sarcoma B cells-Multi-Centric Castleman’s Disease</td>
</tr>
</tbody>
</table>
HSV Lifesytles

A. Establishment of latent infection

1. Virus penetrates into skin, where it replicates.

2. Virus enters cutaneous neurons and migrates to a ganglion, where it remains in a latent state.

B. Reactivation of latent virus

3. Virus can subsequently be reactivated and travel through sensory neurons to the epidermis.

4. A recurrent infection results.
The trigeminal nerve is the major site of HSV-1 latency in mice and humans.
Comparative seroprevalence of HSV-1 and 2 in the US

Looker and Garnett *Sex Transm Infect* (2005) 81: 103-7
HSV virion protein (VP)-16: a paradigm for promoter-specific transactivation in viral replication

HSV IE promoters

IE genes

Transactivators

Early genes

Proteins for vDNA replication

vDNA rep

Late genes

Structural Proteins

HSV IE transcription

TAATGARAT

Oct-1

VP16

HCF-1
Histone modifications are critical in the latent to lytic switch of HSV

Establishment/maintenance of HSV latency

Epithelial cell

a
- VP16 and HCF localize to nucleus
- IE genes expressed

b
- VP16 and ICP0 reduce heterochromatin formation

c
- Genome associates with euchromatin

Neuronal cell

a
- VP16 and HCF in cytoplasm
- IE genes repressed
- LAT expressed

b
- LAT promotes heterochromatin formation

c
- Genome associates with heterochromatin

Productive infection

Latent infection

Varicella-Zoster Viruses (VZV)

VZV gives rise to two distinct clinical syndromes

Varicella = Chicken pox
Primary infection

Latency in ganglia

Zoster = Shingles
Reactivation
Chicken pox
VZV Disease Mechanism

- Transmitted by respiratory route
- Latency in neurons
• ~ 1 million cases/year in US
• > 65 year old individuals
• Reactivation of VZV from dorsal root ganglia - viruses transport to skin
• Occurs only in persons who have previously had chicken pox
• Symptoms:
  A rash that develops into clear blisters (full of infectious virus) and moderate to severe pain, potentially dangerous in the elderly
Epstein-Barr Virus (EBV)

- 1958- first described in a childhood tumor by Dr. Burkitt
  Burkitt’s lymphoma
- 1964- identified by Epstein and Barr by EM
- 90-95% of adults show evidence of EBV infection
Primary Infection

Epith.

Mononucleosis

Latency and Reactivation

Lymphomas (occasional)

Memory B cell

Latent Ag specific

Lytic Ag specific

1° CD8+ T cell response

Memory CD8+ T cell response

Latent Ag specific

Lytic Ag specific
Maintenance of EBV latency and transformation of B cells requires the same proteins.

1. To maintain a latent infection, EBV must stimulate growth and survival of host B cell. This can lead to lymphoma.

   Three EBV proteins are essential for latency/transformation:
   - **EBNA-2 (EBV nuclear antigen-2)** - a transcriptional activator that orchestrates latent gene expression
   - **LMP-1 (latent membrane protein-1)** - can transform permanent cell lines and induce B-cell lymphomas in transgenic mice.
   - **EBNA-1** - required for replicating the latent episomal genome, tethers genome to host chromosome.

2. c-myc is overexpressed by one of two mechanisms:
   a. **EBNA2** directly transactivates it
   b. C-myc is translocated adjacent to a strong cellular promoter.

3. NF-kB is constitutively activated by **LMP-1** - inhibits apoptosis.
LMP1, the major EBV oncoprotein, mimics constitutively active CD40 receptor to activate growth, division, of infected B cells
Kaposi's Sarcoma-Associated Herpesvirus (KSHV or HHV-8)

- KS was first described by Moritz Kaposi in the 1870s
- KS is a cancer that develops in lymphatic endothelial cells
- KS is more common in AIDS patients
  - 91,000 persons with AIDS (1989), 15% have KS
  - >20,000X more common in person with AIDS
  - ~300X more common in other immunosuppressed groups
- A sexually transmitted factor other than HIV plays a role in KS
  - KS is 10X more common in homosexual or bisexual men
- 1994, using a PCR-based technique Chang and Moore identified two small DNA fragments present in AIDS-KS samples - homology to EBV
- 4 clinico-epidemiologic forms: KSHV is etiologic agent of all.
Kaposi’s sarcoma in patients infected with HIV-1

Red, blue or purple flat or raised lesions.
Primary Effusion Lymphoma cells: *The* tissue culture system to study lytic reactivation of KSHV

KSHV is the etiologic agent of
Primary Effusion Lymphoma (PEL)

B cell lymphoma--
B cells are KSHV reservoir *in vivo*

**PEL Cell Lines**
Explanted from PEL patients
50 copies KSHV per cell genome

**Latency**
Virus is latent in >95% of cells
Highly restricted viral gene expression
(ca. 6 genes expressed)
Little spontaneous reactivation
In PEL cells, KSHV is reactivated by chemicals or by ectopic expression of the KSHV ORF50/Rta protein.
TPA induces expression of Rta, leading to a cascade of viral gene expression.

Does Herpesviral reactivation follow a gene expression cascade similar to that of de novo lytic infection?
Rta is an inefficient reactivator

<table>
<thead>
<tr>
<th></th>
<th>ORF59</th>
<th>Rta/59 overlay</th>
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<tbody>
<tr>
<td>Rta</td>
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<tr>
<td>K8.1</td>
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<tr>
<th></th>
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<td>K8.1</td>
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</tbody>
</table>
Mta is a commitment factor for reactivation
Pathogenesis?:

- **KSHV encodes many homologues of cellular growth-control proteins. Most are expressed after reactivation.**

  - **vIL-6:** cell growth
  - **vBCL2:** anti-apoptosis
  - **vIRF:** inhibition of IFN signaling
  - **vCYC:** cell cycle control
  - **vGPCR:** (IL-8 receptor homolog) cell growth
  - **vCCLs:** (chemokines) immune modulation
Sub-optimal progression of KSHV reactivation probably contributes to pathogenesis.

Mta, others?

DE genes
1. vDNA replication proteins
2. Commitment factor

vDNA repl

Late genes
Structural Proteins
K8.1

Lysis of Host Cell
Mature Virus

Rta

DE oncogenes
Pro-growth viral proteins

vGPCR
vIL-6

Lymphatic Endothelium
Kaposi’s sarcoma (KS)

Oct
The host immune response tempers herpesviral reactivation and pathogenesis.

- In immunocompromised hosts (AIDS, transplant, some elderly) herpesvirus reactivation ↑, viral load ↑, and risk and severity of disease ↑.
- Can often be reversed by restoring immunity.
Role of immune system--modeling gamma-herpesvirus infection in the mouse (MHV-68)

Murine Herpesvirus-68

WT Mouse
Long-term latency
HEALTHY MOUSE

WT Mouse
+ Cyclosporine A
LYMPHOMA

IFNγR -/- Mouse
Acute Infection, no latency
DEAD MOUSE

NOSE

Epithelial cell

B cell
## Role of immune system--modeling gamma-herpesvirus infection in the mouse (MHV-68)

<table>
<thead>
<tr>
<th></th>
<th>ΔCD8+</th>
<th>IFNγ −/−</th>
<th>ΔMHC II</th>
<th>Autologous Primed CD4+</th>
<th>ΔB cells</th>
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<tbody>
<tr>
<td># of latently-infected cells</td>
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<tr>
<td>Lytic/Latent Infection</td>
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<td>Reactivation efficiency</td>
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<td>Longer period of reactivation</td>
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Assignment for Next Week:

Oral presentation of two papers on KSHV infection in humans