Mechanisms of Viral Persistence
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Mechanisms of viral persistence

Viral persistence is defined as the inability of the immune system to clear an acute viral infection

- Because of viral mechanisms that circumvent a normal immune system
- Because of immunodeficiency
  - HIV positive individuals
  - Transplant and chemotherapy patients
  - Genetic conditions
  - Congenital infections (tolerance)
A compromise between death and resolution of a viral infection
Mechanisms of viral persistence

A persistent viral infection may cause

- Nothing, supposedly (e.g. polyomaviruses, herpesviruses, torque teno virus)
- Chronic or progressive disease (e.g. measles encephalitis, chronic hepatitis)
- Cancer
- Episodes of clinical reactivation (e.g. genital herpes, infections in the immunosuppressed)
Mechanisms of viral persistence

Definitions

**Persistent infection:** The virus persist in the organism after the acute phase. How long? 7 weeks, 7 months, 7 years, for life.

**Chronic infection:** It implies that there are chronic symptoms of the viral disease (e.g. chronic hepatitis).

**Progressive infection:** Same as chronic, but the symptoms worsen eventually leading to death (measles encephalitis, AIDS)

**Latency:** The virus sits in the organism without signs of replication (herpesviruses). It implies that there is a molecular mechanism to establish and keep the latency.

**Reactivation:** The virus causes a clinical disease (or just replicates) after a period of latency (herpesviruses)
Mechanisms of viral persistence

Viruses that establish lifelong infection in humans

- All human herpesviruses establish latency for life. Some, like HHV6, HHV 7 and EBV, infect virtually everyone.
- Polyomaviruses BK and JC establish an asymptomatic persistent infection in the tubular cells in the kidney for life.
- Torque-tenoviruses (TTVs) – life long asymptomatic persistent infection with shedding
- HIV – integration for life
- HTLV-1 and -2 – integration for life.
Viruses with a specific mechanism for establishing a persistent or chronic infection

- Hepatitis B virus – persistent or chronic infections
- Hepatitis C viruses – persistent or chronic infections
- Human papillomaviruses mucosal and cutaneous – persistent infection, cancer
- Ebola virus - semen

Viruses that occasionally establish a persistent or chronic infection in humans

- Measles– Subacute Sclerosing Panencephalitis (SSPE)
- Rubella – congenital, Fuchs uveitis, granulomatosis
- Many viruses in the immunosuppressed patient
- Ebola virus – reactivation?
Viral infection

- Incubation
  - The virus must overcome the innate immune system

- Acute viral replication
  - Clinical disease (or not)

- Persistent Infection
  - The virus must overcome the adaptive immune system

Death or resolution

Mechanisms of viral persistence
The innate immune responses
Innate (intrinsic) immune system. The main pathways.

1. Toll like receptor (TLRs) → Inflammatory cytokines → adaptive immunity → Interferons type 1 → innate antiviral response

2. RNA helicase Rig-I-like receptors (RLR) → same response as TRLs

3. Type I and III IFNs → PKR system induction → OAS system induction → Activation on NK and cytotoxic T cell → Enhancement of antigen presentation to T cells

4. PKR System → Inhibition of translation → Apoptosis → NFkB activation

5. Autophagy → Destruction of infected cell → Enhancement of antigen presentation

6. Ag presentation → T cell response
Mechanisms of viral persistence

Toll-like receptors

Plasma membrane

Endosomes and other cytoplasmic membranes

Mechanisms of viral persistence

RNA helicase Rig-I-like receptors (RLR)

Takeuchi and Akira. Cell. 2010; 140:805
Many viruses inhibit the TLR and RLR pathways

From Hiscott et al. Oncogene. 2006; 25:6844-6867
The PKR system and its inhibition by viral proteins

From: Garcia et al. Biochimie 2007; 89:799-811
Mechanisms of viral persistence

Autophagy and its regulation by viral proteins

From: Jordan and Randall. Microbes and Infection 2012; 14:126
MHC Class 1 – All cells

MHC Class 2 – “Professional” Ag presenting cells

From: The Swiss Institute for Bioinformatics
Adaptive immune response
Adaptive immune response against viruses

- Production of specific IgM – in acute infection. A week after contact with the virus – Stimulate phagocytosis and Ag presentation
- CD4+ and CD8+ T cells — Help the production of Ab, destroy infected cells
- Production of IgG – Phagocytosis and destruction of the agent
- Production of neutralizing IgG – prevent spreading and re-infection
After evading the innate immune system, viruses must survive the adaptive immune system.

Nature Reviews Immunology 9, 741-747
Specific strategies of viral persistence
General mechanisms of persistent infection


2. Modulation of the adaptive immune system – e.g. herpesviruses, HIV, measles

3. Evasion from the immune system by latency in a long-lived episomal (e.g. herpesviruses, hepatitis B) or an integrated (e.g. retroviruses) state

4. Infection of tissue not readily accessible by the immune system (e.g. Human papillomavirus, molluscum contagiosum)

5. Tolerance – in congenital infections (HCMV, rubella, hepatitis B)

6. Saturating the immune system – hepatitis B surface antigen

7. Prolonging the survival of the infected cell – e.g. oncogenic viruses

8. Immune response escape mutants (e.g. hepatitis C, HIV)
Hepatitis C virus

Family Flaviviridae
Genus Hepacivirus

RNA virus
Replication and assembly on the endoplasmic reticulum
Hepatitis C – A typical chronic viral infection

- After the acute infection, usually asymptomatic, only about 20% of patients clear the virus after a few months or years.
- The rest go on to a chronic life-long disease, with viremia and various degree of inflammation that may lead to cirrhosis or cancer.
HCV mechanisms and factors of persistence

- HCV protein NS3/4A and NS5A/E2 inhibit the innate response
- Genetic variation of the virus
- Inhibition and evasion of the adaptive immunity
- Genetics of the host.
Innate response to HCV infection

- **NS3/4A protease** inhibits both RIG-1 and TLR3 responses by cleaving key pathway proteins.
- **NS5A/E2** inhibits the PKR response.
Is this mechanism clinically relevant for HCV persistence?

Most likely:
Interferon alpha therapy (with ribavirin) is effective in clearing the virus.

Genetic diversity of the virus is also a factor.

Genotype 2 is cleared by IFN-alpha + ribavirin in 80% of cases
Genotypes 1 and 3 are cleared in only 50% of cases
Evasion of the adaptive immune response

- Both antibody and CD4+ and CD8+ lymphocyte immune response is delayed to 6 to 8 weeks post-infection. The mechanism is unclear.

- Early Ab and CD4+ and CD8+ response is associated with clearance of the virus

- Mutational escape reduces the effectiveness of neutralising Ab
- Mutational escape reduces the effectiveness of CD8+ cells
- CD8+ cell receptor function is reduced (viral mechanism is unknown)
- CD4+ cell function is reduced (inhibition of interferon response)
20-30% of infection are cleared spontaneously

A variant of the IFNL4 gene is strongly associated with chronic infection and with success of interferon therapy.

Success of IFN therapy in African Americans is only 30%. Unclear if this is connected with the IFNL4 variant.
Human Papillomavirus
Hiding from the immune system

- Infection of keratinized epithelia, which have very few “professional” Ag presenting cells.
- Normally an infection lasts 6 months, much longer in a minority of cases.
- Convincing inhibitory mechanisms of the immune systems have not been demonstrated in HPV infection.
- HPV survives by keeping the infected cells alive by mitogenic molecules E6 and E7.
Immunomodulation

“Virokines” – Viral gene products which mimic the function of cytokines, usually with an inhibitory effect on the immune system. Many virokines are homologous to the host cytokines:

- HCMV
- EBV
- HHV8
- HHV6 and 7
- Poxviruses

The end result is an equilibrium between immune system and viral replication which maintains a persistent infection or latent stage.
Alignment of human IL-10 and EBV BCRF-1

90% homologous to human IL-10.

Inhibits NK and CD8 response toward EBV-infected B lymphocytes
Inhibits interferon response but not B cell proliferation.

Typical example of gene acquired by herpesviruses from the host
Human cytomegalovirus (HCMV)

- A herpesvirus.
- Large (230kb), complex (208 ORFs), ds DNA genome.
- *In vivo* it infects mucosal cells in the oropharynx, myeloid cells and granulocytes, monocytes and macrophages.
- Does not cause disease in healthy individuals.
- After primary infection establishes life-long latency in myeloid precursors.
- Subclinical reactivations in oral mucosae ensure transmission through saliva.
- Reactivation in immunosuppressed individuals cause retinitis, pneumonia, generalized infection.
- Primary infection of pregnant women results in congenital infections.
Reaching an equilibrium with the host adaptive immune system

1. Down-regulation of MHC Class I
   gpUS3, gpUS2, gpUS11 reside in the ER and direct the degradation of newly formed MHC I complexes.

2. Inhibition of Ag presentation.
   gpUS3, ppUL83 inhibit peptide translocation to the MHC

3. Inhibition of IFN-mediated MHC class II response
   mechanism unknown

4. Suppression of NK and CD8 activity.
   UL111A is an IL-10 homologue

5. Other homologues of human genes of uncertain function
   UL18 - MHC I heavy chain homologue
   UL33, UL78, US27, US28 - Homologues of chemokine receptors
   UL148 - homologue of IL-8
   UL144 - homologue of TNF
   • and others
Congenital infections

Usually, maternal immunity and the placental barrier prevent viral infection of the foetus. When infectious happens the foetus does not mount an efficient immune response and it may become tolerant to the virus.

**HCMV congenital infection** – During primary infection of the mother. Severe congenital defects and shedding of HCMV at birth.

**Rubella congenital infection** – During primary infection of the mother, especially in the first trimester. Congenital defects, persistent infection with rubella virus that may last for a year after birth. Slow maturation of the antibodies.

**Hepatitis B congenital infection.** From a chronically infected mother to the foetus. Almost complete tolerance with infection for life.
HSV-1
Genomic organization of simplexviruses

LAT region

LAT region

LAT region

LAT region

LAT region

LAT region

LAT region
First infection: stomatitis

From Fields Virology
Recurrent herpes

From Fields Virology
Herpes encephalitis

From Fields Virology
Establishment

- Establishment occurs in the sensory ganglia and to less extent in brain cells (human and animal models).
- Ten to 100 copies of the circular genomes (no ends) sit in the cell nucleus.
- Establishment of latency occurs by default (animal models), i.e. none of the genes tested using mutants is essential (except LATs). Replication is not required. Gene expression is not required.
- Sort of a latent phenotype can be obtain in culture by heat shock and by low multiplicity infection with an ICP0 null mutant.
<table>
<thead>
<tr>
<th>Herpes simplex</th>
<th>Varicella</th>
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</thead>
<tbody>
<tr>
<td>Mild pharyngitis fever</td>
<td>Chickenpox</td>
</tr>
<tr>
<td>Fever</td>
<td>Age and X-irradiation (act via depressed CMI)</td>
</tr>
<tr>
<td>Sunlight to face</td>
<td>Local injury or infection (e.g. sinusitis)</td>
</tr>
<tr>
<td>Menstruation</td>
<td>Nerve section at *</td>
</tr>
<tr>
<td>Nerve section at *</td>
<td>Activation of virus in neuron</td>
</tr>
<tr>
<td>Cold sore</td>
<td>Zoster (shingles)</td>
</tr>
<tr>
<td>Virus transit down peripheral nerve</td>
<td>Spinal cord</td>
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Fig. 10.1 Mechanism of latent herpes simplex and varicella-zoster virus infection in man.
HSV in nerve axons
Mouse model for latency

Murine Eye Model for HSV Latency and "Reactivation"
(N. Sawtell and R. Thompson, U. Cinn.)

- HSV
- Viral genomes recovered in trigeminal ganglia
- 2 to 3 weeks
- Survives
- Trigeminal ganglia plus indicator cells
- Animal sacrificed
- PCR
- Infectious virus recovered
- cell culture

Variable mortality
Maintenance

• Like for establishment none of the genes tested using mutants is essential. Replication is not required.

• Some replication and, therefore, gene expression must occur at least at the time of reactivation.

• The only transcripts detected are the LATs (latency associated transcripts, especially the 2kb stable intron

• All evidence was obtained in animal models
Latency Associated Transcripts (LATS)

Stable intron
Role of LATs

- The main LATs transcript is antisense to ICP0. The attractive hypothesis that LATs act as a post-transcriptional repressor of ICP0 has not been confirmed.
- LAT-negative mutants do not reactivate efficiently, but reactivation is never abolished (animal models). It is hard to produce LAT mutants without affecting ICP0.
- Recently LATs have been shown to inhibit apoptosis in neurons (animal models).
- The function of LATs could be to preserve infected the neurons for reactivation.
Apoptosis inhibition is required for latency (or for reactivation?)


LAT deleted + Baculovirus antiapoptotic protein

wt

LAT deleted
An miRNA, and not the stable intron, may be the anti-apoptotic factor of LATs

Gupta et al. (2006) Nature 442:82-85
Role of the immune system in HSV1 latency and reactivation

Clues that the immune system may be involved:

- Traditional triggers of HSV reactivation (UV light, “stress”, concomitant infection) are known depressor of local or systemic cell-mediated immunity.
- In natural infection, reactivation frequency decreases with time
- Immunosuppressed patients are much more likely to have frequent and severe reactivation, including systemic infection
- For other herpesviruses (VZV, EBV, CMV, HHV-6, HHV-8) immunosuppression is basically the only cause of reactivation
- It has been shown in the mouse model that HSV-specific CD8+ T cells infiltrate the trigeminal ganglia and cluster around HSV-infected neurons
- The number of infiltrating CD8+ cells correlates inversely with the rate of reactivation
Hepatitis B virus

- Partially dsDNA genome (open circular), about 3,000 bases
- Overlapping ORF
Hepatitis B – multiple mechanism for persistence

1. Stage of long lasting covalently-closed-circular (ccc) episome in long lasting cells (hepatocytes)
2. Overproduction of circulating surface Ag competes out IgG antibodies and prevents tagging hepatocytes for cytotoxicity
3. Congenital infection
4. Host genetics?
Hepatitis B virus persist as a rule
An evolutionary ecosystem of persistent viruses

A different angle on viral infections
• Retroviruses integrate in the host genome upon entry in the cell.
• HIV is deadly, but others like HTLV-1 and 2, rarely cause diseases.
• A great number of endogenous retrovirus sequences inhabit our genome
• There is evidence that some of these sequences are functional.
“Endogenous” retrovirus reactivating in the mouse

Young GR et al. Nature. 2012; 491:774-8
Herpesvirus evolution and retroviruses

- HHV6 infects nearly 100% of adults
- HHV7 infects nearly 100% of adults
- EBV infects 90% of adults
- CMV used to infect 90-100% of adults (now about 50%)

- They all establish latency for life in immune system cells
- These viruses diverged in evolution about 100 millions ago
- They all contain a variety of homologues of human immune genes. Without introns. cDNA.
The end