The problem of latent virus for treatment and prevention of human cytomegalovirus

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• Human cytomegalovirus (CMV) impact on the community
• Three phases of infection
• Focus on the dormant (latent) phase of infection
• Why doesn’t our immune response prevent/clear latent infection?
• What are the implications of latency for therapies to prevent CMV disease?
Human cytomegalovirus: impact on the community

- Member of the Herpesvirus family.
- Large double stranded DNA genome encoding >200 genes
- Highly species and cell-type specific
- High seroprevalence, which varies geographically around the world from 45-100%

- The good news:
  Most infections are mild or asymptomatic in healthy people
• The bad news: Causes devastating disease in two main at risk groups

(1) Unborn babies infected during pregnancy

• CMV is the most common virus transmitted to the unborn child

• Approx 1 in 150 children is born with congenital CMV

• Approx 1 in 750 children is born with or will develop permanent disability as a consequence of CMV
Congenital CMV disease is common

- Congenital CMV is a more common cause of serious disability than Down Syndrome, fetal alcohol syndrome and neural tube defects.
(2) People with weakened immune systems

**HIV AIDS**

- The era of solid organ transplantation, immunosuppressive cancer chemotherapy, and of AIDS highlights the importance of CMV disease immunosuppression

- **High mortality**: Eg even treated with antiviral drugs, the mortality of allogeneic stem cell patients is 15-75% (drug toxicity and late CMV disease once ceased)

- **Increased health care costs**: Eg in solid organ recipients with CMV disease, the number of days in hospital more than doubles and costs increase by >30%

- US National Academy of Sciences Institute of Medicine has assigned the highest priority to the development of a CMV vaccine

- CMV disease in transplant patients due to reactivation of virus from a latent state
Phases of CMV infection

1. Productive Infection
   - Extensive viral gene expression
   - Extensive replication
   - Causes cell death

2. Latent Infection
   - No replication or new infectious virus
   - Life long

3. Reactivation
   - Clinically important
   - Can cause life threatening disease

Despite the major importance of latency/reactivation to the success of the virus as a human pathogen, these phases of infection remain very poorly understood.
Targeting latent infection

• The capacity of CMV to establish, maintain and subsequently reactivate from latency ensures its widespread dissemination in the community

• This accounts for much of the life-threatening infectious disease in solid organ and allogeneic bone marrow transplant recipients
Effects of targeting latent infection

• Clinical benefits of elimination of the latent pool of CMV infection:
  – Direct benefit to those at risk of disease arising from virus reactivation from latency
  – Benefit to those at risk of disease arising from primary CMV infection by reducing the incidence of infectious CMV circulating in the population due to reactivation from latency
Site of latent infection

- Myeloid lineage cells are the predominant site of latent infection.
- Reactivation occurs when myeloid progenitors differentiate to myeloid DC or macrophages.
Why don’t anti-viral drugs clear the latent virus?

• Current antiviral drugs all target the virus during its replicative cycle (either viral DNA synthesis or cleavage of newly replicated viral DNA)
  – Ganciclovir (GCV)
  – Valganciclovir (VGCV)
  – Foscarnet (FOS)
  – Cidofovir (CDV)
  – Letermovir (in trials)

• Latent CMV present at about 2-15 viral genomes per latently infected cell (Slobedman and Mocarski, 1999, *Journal of Virology*)

• Latent CMV genome is present as in circular, extrachromosomal configuration (Bolovan-Fritts et al., 1999, *Blood*)

• No evidence that CMV replicates during latency
Why doesn’t our immune response prevent or clear latent CMV?

• Healthy humans elicit a massive T cell response against CMV
  – ~5% of total CD4+ and CD8+ T cells are CMV-specific (this accounts for ~10% of the total memory T cell compartment)

• This T cell response is also remarkably broad
  – Out of 213 CMV ORFs studied, 151 elicited a CD4+ and/or CD8+ T cell response (Sylwester et al., 2005, *J Exp Med*)

• During CMV latency, cytotoxic CD4+ T cells also emerge (van Leeuwen et al., 2006, *Blood*)

• CMV-specific T cells increase further during aging (20-50%), which may contribute to immune senescence (Mekker et al., 2012, PLoS Pathogens)

Why doesn't this huge, anti-CMV immune response clear the latent virus?
Why doesn’t our immune response prevent or clear latent CMV?

• Issue compounded by the fact that CMV establishes a life-long latent infection in myeloid cells which are, or will become, potent antigen presenting cells

• Question: How is CMV able to remain latent in these cells in the face of a robust immune system?
Viral gene expression during latency

- Are CMV-specific T cells unable to recognise latently infected cells because latent CMV does not express any viral genes?
### Viral gene expression during CMV latency

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Out of ~200 viral genes, 35 were identified as being expressed during the establishment and maintenance of latency in myeloid progenitor cells.


Immune control during CMV latency

- Does CMV actively control the host immune response to facilitate long term latency?

- **Hypothesis:** CMV is likely to orchestrate an environment in latently infected cells that enhances its ability to persist within the human host by regulating host immune response genes

- Identified a viral gene which expresses a homologue of the potent immunosuppressive cytokine IL-10 (Jenkins et al., 2004, Journal of Virology)

- Latent viral IL-10 encoded by the CMV UL111A gene
Control of T cell recognition by CMV encoded viral IL-10 during latent infection

- Viral IL-10 expressed during latency suppresses MHC class II expression by latently infected cells to render these cells refractory to CD4+ T cell recognition

Slobedman et al., 2009, *Journal of Virology*
Jenkins et al., 2008, *Journal of Virology*
Slobedman et al., 2002, *Blood*
Cheung et al., 2009, *Blood*
Control of differentiation of latently infected myeloid progenitor cells by viral IL-10

- Viral IL-10 expressed during latency skews differentiation of latently infected cells away from becoming dendritic cells (Avdic et al., 2011, *Journal of Virology*).

- Thus, viral IL-10 not only enables latently infected cells to evade CD4+ T cell recognition, but also functions to modulate differentiation of latently infected cells away from the most potent antigen presenting cell type (DC).
CMV control of the host microenvironment during latency

- Besides viral IL-10, latently infected myeloid cells secrete other factor(s) that modulate the microenvironment.

- Latent CMV induces chemokines MCP-1 (Stern and Slobedman, *Journal of Immunology*, 2008) and MCP-2 (Mason et al., 2012, *PNAS*).

- Latent CMV firstly recruits CD4+ T cells by upregulation of MCP-2 and then inhibits their antiviral functions by also upregulating human IL-10 and TGF-β (Mason et al., 2012, *PNAS*; Slobedman and Mocarski, 2012, *PNAS*).
Targeting latent CMV immune evasion functions

- Latent CMV is far more active during latency (viral genes are expressed)
- Latent CMV manipulates the host cell/environment in such a way as to limit the effectiveness of anti-viral T cell responses
- At least one CMV gene product expressed during latent infection (viral IL-10) actively evade immune mediated clearance of latent virus.
- Viral IL-10 therefore serves as a target for the development of drugs that could block the capacity of latent virus to evade immune detection (enabling existing CMV specific T cells clear the latent pool)
CMV latency and vaccination

- Multiple approaches currently being undertaken to develop a vaccine against CMV
  - Live, attenuated viruses
  - Subunit
  - Vectors
  - DNA vaccines

- Boosting immunity to CMV in those already latently infected with CMV is unlikely to impact on the latent pool of virus, but it may reduce the severity/frequency of reactivation (ie viraemia in transplant recipients)

- Some encouraging results:
  - gB subunit vaccine approach (Sanofi Pasteur) Phase II trial. Compared to placebo, solid organ (kidney, liver) seronegative recipients from seropositive donors had reduced duration of viraemia (Griffiths et al., 2011, Lancet)
  - DNA vaccine approach (TransVax: gB+pp65; Vical) Phase II trial: occurrence of CMV reactivation in stem cell transplant recipients reduced (33% versus 62%) (Kharfan-Dabaja et al., 2012, Lancet Infectious Diseases)
Remaining challenges/questions

• The relative contributions of re-infection and reactivation from latency during pregnancy to congenital CMV infection are still not fully understood (Griffiths et al., 2013, *Vaccine*).

• Will vaccination prevent latency? Not clear
  • Vaccination with live, attenuated vaccine (Towne) did not prevent subsequent productive infection (and presumably latency) (Plotkin et al., 1994, *Transplantation*).

• The full repertoire of viral genes which function to evade immune clearance of latently infected cells is not yet known.

• The full repertoire of immune evasion functions of already-identified viral genes which are expressed during latency is not yet known.

• Study of human CMV latency is hampered by high species and cell-type specificity (models of latency are complex).

• A much better understanding of the molecular determinants of latency and reactivation is required to inform the development of novel therapies to target establishment, maintenance or reactivation phases of latency.
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