Immunity to cytomegalovirus....and why you should care

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Persistent viral infection

- It’s a two party battle: host offense vs viral defense (we try to look at both sides)

- How do the TNF-family cytokines and cosignaling pathways regulate the immune ‘balance’ that develops over a lifelong infection.

- Herpesviruses are well suited to ask these questions. They establish a lifelong relationship with their hosts, which in the case of CMV and others, requires maintaining immunity to repress recurrence/disease.
Cytomegalovirus (HHV-5, β-herpesvirus)

Some Facts:

- dsDNA genome of ~230kB

- Establishes a lifelong, latent/persistent infection (as do all the α, β & γ herpesviruses)

- ~30-90% of the USA population is infected: varies by age, geography, socioeconomic status. Appears that infection rates are going down in several developed countries.

- Transmitted largely by fluid transfer: breast feeding, sheds in urine/saliva at high levels (can do so for years, asymptotically, in young kids).

- Infection of immune competent persons (even neonates) is largely asymptomatic. Causes disease upon infection of immune suppressed & compromised (e.g. transplant recipients & AIDS patients) and naïve (congenital).

- Institute of Medicine lists it as a high-priority for a vaccine. Costs USA health care system ~5 billion $$ annually.
Cytomegalovirus has an image problem......

(It is a significant public health risk, but few people know it)

- Very little public awareness of the clinical issues caused by CMV. Clinicians in transplant units known about it...and maybe OB/GYNs.
  ♦ “Doesn’t cause a rash”...VZV & HHV6/7 (Roseola)
  ♦ They didn’t tell us it’s ‘forever’ like I learned in health class for HSV
  ♦ Really? I can get it by kissing someone?? (like EBV ‘mono’)

- ~1 in 750 fetuses born in the USA have disease due to congenital CMV infection (largely hearing loss & cognitive disorders)
  ~10x this many fetuses get infected, but have no clinical symptoms.
    sero(-) pregnant women with primary HCMV infection = highest risk group

- Transplant recipients (HSC, BM & solid organ) can get high levels of HCMV viremia, can lead to potential rejection and/or severe disease (e.g. pneumonia). Commonly treated with antivirals (e.g. Valganciclovir & Foscarnet), but they can be quite toxic.

- Several diseases potentially are a result, or amplified by, chronic HCMV infection:
  ♦ Strongest links for: CVD, all-cause mortality & immune senescence (NHANES)
  ♦ Tumors (glioblastoma).
  ♦ HCMV ‘occupies’ so much of our T cell memory pool, perhaps this contributes?
Cytomegalovirus vaccine development

- Has been a high-priority issue of the IOM for ~13 years.

Recent NIH workshop where at least 5 major pharmaceutical companies

My bias: Any vaccine approach will be greatly facilitated by inducing durable HCMV-specific CD4 T cells, and also likely CD8 T cells.

Cellular immunotherapy using HCMV specific T cells protects against disease in BM transplant. CD4 + CD8 is best (Riddell & Greenberg)

Something to remember: HCMV can reinfect a pre-immune host, so this makes the prospect of developing a ‘sterilizing’ vaccine very challenging

EM cells that correlates with congenital protection (not CD8T or Ab).

Gerna et al., JID 2008
CMV can teach us a lot about host immunity

(McGeoch, 2000)

HCMV genome ~235kB

Most the conserved/essential orfs in the various CMVs

MCMV genome ~230kB

Loewendorf and Messerle

Δ8 Δ1 Δ9 A4

Δ6 Δ7 Δ1 A2 A3 A1

Loewendorf and Messerle

MIEP GFP gpt BAC

Δ1 Δ8 Δ9 A4

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Loewendorf and Messerle

MIEP GFP gpt BAC
Course of CMV infection in immune competent mice

Innate Immune Response
- IFN-αβ
- NK
- iNKT

Adaptive immune response
- CD4 T cells
- CD8 T cells
- IFN-γ
- Perforin

Days Post Infection
- d3
- d7
- d14
- d60
- d600

MCMV Replication

Salivary Gland

Most visceral organs

Sporadic reactivation?

Arens et al, 2008

"Inflationary" memory CD8 T
(Reddehase, Klenerman, Hill)
MCMV epitope-specific CD4 T cells (I-A$^b$)

Arens et al., 2009

Days Post MCMV Infection

%IFN-γ + CD4 T cells

A

m18

M45

M25

M45

M104

M122

m139

m139

m141

m142

m142

no peptide

PMA/iono

TNF

IL-2

IL-10

IL-17

% cytokine

IFN-γ

CD4

%IFN-γ + CD4 T cells

Days Post MCMV Infection

Arens et al., 2009
MCMV-specific ‘inflationary’ CD4 T cells depend on the salivary gland, and have a unique memory phenotype

Splenic CD4 T cells analyzed at day 100 after infection

[Bar chart showing the number of IFNγ+ CD4 T cells with and without salivary gland removal for m142 and m09 strains]
Cosignals are critical in regulating T cells

- **CD8 T**
- **CD4 T**

**Signal 1**
- TCR
- **“signal 1”**

**Signal 2**
- Cosignals
- Co-Inhibitory (−) “signal 2”
  - HVEM ---- BTLA
  - PD-1 ------ PD-L1
  - B7.1/2 ---- CTLA-4

**Co-Stimulatory**
- B7.1/B7.2-------CD28
- OX40L ------- OX40
- 4-1BBL ---------- 4-1BB

**Signal 1 + Signal 2**
- Cytokines (IFNγ, TNF, etc)
- Cytolytic activity
- Proliferation
B7-CD28 costimulation is required for MCMV-specific CD4 T cells

CD4T control replication in the salivary gland...CD8T exert no control here!
B7-CD28 not required for CD8T memory inflation

**M45**

IFN$\gamma$+ splenic CD8$^+$ T cells ($\times 10^6$)

**m139**

IFN$\gamma$+ splenic CD8$^+$ T cells ($\times 10^6$)

**m38**

IFN$\gamma$+ splenic CD8$^+$ T cells ($\times 10^6$)

**IE3**

IFN$\gamma$+ splenic CD8$^+$ T cells ($\times 10^6$)
MCMV inhibition of B7-1 and B7-2 by m138 and m147.5 restricts CD4 T cells and promotes persistent replication

Take Home: CD8T are being primed by uninfected cells (i.e. cross-primed)
PD-L1 is not downregulated in MCMV infected dendritic cells

Infected with MCMV-GFP (MOI=2), gated on GFP+ cells for FACS analysis
Does not bind LIGHT

HCMV UL144

HCMV genome ~235kB

Cha et al., 1997

orf UL144 (a HVEM orthologue)
LIGHT-HVEM-BTLA network is targeted by herpesviruses.
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