Viruses, Cells and Disease
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Human Cytomegalovirus (HCMV)
Immediate early proteins, gene expression and signaling

Dr. Hua Zhu
ICPH E350D
UMDNJ - New Jersey Medical School
973-972-4483 X 2-6488
zhuhu@umdnj.edu

Electron Cryomicroscopy and 3D Reconstruction
From Dr. H. Zhou
Introduction
1. Medical aspects of HCMV
2. HCMV structure
3. HCMV replication

Special topics
1. IE1 and IE2 functions
2. A HCMV-induced signal transduction pathway
   (discuss two papers next week)
3. Functional profiling of HCMV genome
4. Identification of HCMV pathogenic genes
**HCMV**

**Virus**
- Herpesvirus, dsDNA virus
- ~80% of adults are infected

**Transmission**
- in utero and at any age
  - body fluids
- breast feeding, blood transfusion
**Latency and Recurrence**
- Latent in monocytes (Myeloid progenitor cells)
- Reactivates during pregnancy and in immunosuppressed states, resulting in cytomegalic inclusion disease (CID), CMV pneumonia, retinitis and hepatitis

**Pathogenicity**
- In people with cancer, transplanted organs, AIDS or other immune deficiencies, CMV can cause severe diseases of the lung, liver, colon, eye or brain
- A leading cause of congenital birth defects
Transplant Recipients
Immunosuppression

Rates of CMV Infection and disease in liver transplant patients according to types of infection

<table>
<thead>
<tr>
<th>Pretransplant serostatus of donor/recipient</th>
<th>Type of infection</th>
<th>Infection (%)</th>
<th>Disease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>Reinfection or reactivation</td>
<td>66</td>
<td>23</td>
</tr>
<tr>
<td>+/-</td>
<td>Primary</td>
<td>77</td>
<td>61</td>
</tr>
<tr>
<td>-/+</td>
<td>Reactivation</td>
<td>49</td>
<td>10</td>
</tr>
<tr>
<td>-/-</td>
<td>Primary, not from graft</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
HCMV and AIDS

- Common immediate cause of death in AIDS (25%)
- 70-90% show active CMV infection
- 30-40% develop CMV retinitis
- 95% homosexual men with AIDS have active HCMV infection

CMV retinitis
Congenital HCMV Infection

- The most common virus globally to be transmitted in utero, affecting up to 2.5% of all live births (WHO)
- A leading cause of birth defects
- ~10% of infected fetuses exhibit damage of the CNS-mental retardation
  hearing loss
  impaired vision
Prevention and treatment

• Vaccines: AD169, Towne - not effective

• Antivirals: Ganciclovir - effective
Anti-Herpesvirus Agents

**Acyclovir** (Zovirax - Glaxo) - for $\alpha$ herpesviruses

**Ganciclovir** (Cytovene - Roche) - for $\beta$ herpesviruses

- Guanosine analogs

**Mechanism of Action:**
- Specifically activated by virus-induced kinases, such as thymidine kinase of HSV and UL97 of CMV
- Terminate viral DNA chain elongation
- Selectively inactivate viral DNA polymerases and inhibit viral DNA elongation
Acyclovir

Viral kinase

Acyclovir monophosphate

Cytosine

Ganciclovir

Viral DNA polymerase

Acyclovir triphosphate

DNA Polymerase

Ganciclovir triphosphate

Deoxyguanosine triphosphate

Chain termination
HCMV Virion Structure

- Icosahedral nucleocapsid
- dsDNA genome
- Envelope
- Tegument
- Glycoprotein
HCMV Genomic Structure

CMV (230kb)

UL

US

AD169
Towne
Toledo
PH
TR
FIX
HCMV Encodes 230-250 Potential ORFs

Murphy et al, 2003
Early Events of HCMV Infection

HCMV gB

HSPG

gH gM/N

EGFR?
Integrins

IE
Overview of HCMV Gene Expression Patterns

Cycloheximide

UL122 (IE2), UL123 (IE1), UL36, UL37, US3, UL119, TRS1 (IRS1)
HCMV Major IE Locus

Major IE promoter

TATA

ATF/CREB
AP1
SP1
NF-1
NF-κB

IE1 mRNA
IE1 protein (72 kD)

IE2 mRNA
IE2 protein (86 kD)
1. **IE1 and IE2 Functions**

Is IE2 essential for HCMV replication?
Construction of HCMV-BAC

- HCMV DNA
- BAC vector

Transfect HF cells

Homology recombination

- Produce infectious HCMV

- Purify recombinant HCMV (Green)

- Mutagenesis
  - Specific
  - Random

- Transform into E. coli
- Select CmR colonies
- Screen full-length HCMV-BAC

- Infect new HF cells
- Isolate circular viral DNA
Generation of Recombinant HCMV

**PCR kan^R^ cassette**
Transform into DY380

Select for kan^R^ colonies at 32°C

Confirm recombinant HCMV-BAC by PCR and Southern

**Incubate at 42°C**
Preparing electro-competent cells

**E. coli** DY380 32°C

Defective λ prophage

**ΔORF mutant**
Isolate BAC DNA

Transfect HF cells

Produce infectious HCMV

Dr. Neal Copeland
IE2 is essential for viral replication

MIEP

TATA

WT

E1 E2 E3 E4 E5

IE2

Δ

Deletion of exon 5

IE2Δ

Genotype | Plaques
---------|--------
WT       | 223
IE2Δ     | 0
IE2 is required for the early and late gene expression.
Do IE1 and IE2 directly regulate viral gene expression?
ChIP-Chip Assay

IE1 → IE2 → TFs → Viral promoter

1. Formaldehyde treat cells
2. Isolate nuclei
3. Sonicate
4. Anti-HCMV IE1/2 Immunoprecipitate (anti-IE1/2)
5. Reverse crosslinks
6. Identify target DNA

or

Anti-HSV gB

Identify target DNA
• IE2 is essential for viral replication
• IE2 is required for viral early and late gene expression
• IE1 and IE2 directly regulate viral gene expression
2. A HCMV-induced Signal Transduction Pathway
What Are the Host Response to HCMV?
Identification of cellular genes altered after HCMV infection

- Differential display
- GeneChip (Affymetrix)
- \textit{cigs} (IFN-stimulated genes, \textit{isgs})
- Activated by both live and killed HCMV
- Not require viral gene expression, viral DNA, and new protein synthesis
- Not mediated by IFNs or other cytokines
- HCMV directly activates ISGs

\begin{verbatim}
cig1  cig6  cig24  cig2  cig3  cig41  cig4  cig43  cig49  cig52  cig5  contro
\end{verbatim}

\textit{cig1} = isg54k
What is required for induction of ISGs?

HCMV

HSPG

gH

gB

HCMVR

IE

ISG
Is fusion required?

HCMV: - W W M M M
CFI: - - + - +

isg54K -
cig49 -
7 SK -

HCMV: - - + + + +
PEG: - + - - +
CFI: - - - + +

isg54K -
7 SK -
3. Functional Profiling of HCMV Genome

No growth: 45; Severe growth: 12; Moderate growth defect: 23; Growth like WT: 68; Enhanced growth: 4

Dunn et al., 2003, PNAS
A: Fibroblast cells; B. Epithelial cells; C. Endothelial cells
4. Identification of HCMV Pathogenic Genes

CMV (240kb)

UL

US

AD169
Toune
Toledo
PH
TR
FIX
The Severe Combined Immunodeficient (SCID) Mice Model For Studying HCMV Pathogenesis

Infected implants are harvested at different time and the amount of HCMV is measured.

A thymus/liver implant is surgically exposed and injected with HCMV.
The clinical but not attenuated strains can replicate in the implanted human tissues

<table>
<thead>
<tr>
<th>Attenuated strains:</th>
<th>Fibroblasts</th>
<th>SCID-hu</th>
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</thead>
<tbody>
<tr>
<td>AD169, Towne</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Clinical strains:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toledo</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Construction of A HCMV with a Luciferase Gene

Luciferase (UL62/UL63)

Luciferase

HCMV_BAC  HCMV_luc

0  5000000  10000000  15000000  20000000  25000000  30000000
Bioluminescence Imaging Technique
*(Xenogen IVIS® Imaging System)*

- A. Virus w/ Luc.
- B. Animal/cell models
- C. Image acquisition
- D. Data analysis

Images of Toledo and Toledo_Luc.
The 15-kb Segment Contains the Crucial Genes for HCMV Replication \textit{in vivo}