Viral Replication

Scott M. Hammer, M.D.

Viral Replication: Basic Concepts

- Viruses are obligate intracellular parasites
- Viruses carry their genome (RNA or DNA) and sometimes functional proteins required for early steps in replication cycle
- Viruses depend on host cell machinery to complete replication cycle and must commandeer that machinery to successfully replicate
Viral Replication: Basic Concepts

• Replication cycle produces
  - Functional RNA's and proteins
  - Genomic RNA or DNA and structural proteins

• 100's-1,000's new particles produced by each cycle
  - Referred to as burst size
  - Many are defective
  - End of 'eclipse' phase

• Replication may be cytolytic or non-cytolytic

Steps in Viral Replication: Attachment
(First Step)

• Surface protein on virus attaches to specific receptor(s) on cell surface
  - May be specialized proteins with limited tissue distribution or more widely distributed
  - Virus specific receptor is necessary but not sufficient for viruses to infect cells and complete replicative cycle
Selected Virus Receptors

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>CAR, CD55, Integrin VLA-2, CD55</td>
</tr>
<tr>
<td>Coxsackievirus</td>
<td>CAR, CD55, Integrin VLA-2, CD55</td>
</tr>
<tr>
<td>Echovirus</td>
<td>CD21, CD4, CCR5, CXCR4, CD46</td>
</tr>
<tr>
<td>Epstein-Barr Virus</td>
<td>CD21, CD4, CCR5, CXCR4, CD46</td>
</tr>
<tr>
<td>HIV-1</td>
<td>CD21, CD4, CCR5, CXCR4, CD46</td>
</tr>
<tr>
<td>Measles virus</td>
<td>Erythrocyte P Ag</td>
</tr>
<tr>
<td>Parvovirus</td>
<td>PVR</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>ICAM-1</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>ICAM-1</td>
</tr>
</tbody>
</table>

Steps in Viral Replication: Penetration (Second Step)

- Enveloped viruses penetrate cells through fusion of viral envelope with host cell membrane
  - May or may not involve receptor mediated endocytosis

- Non enveloped viruses penetrate by
  - Receptor mediated endocytosis
  - Translocation of the virion across the host cell membrane
Steps in Viral Replication: Uncoating
(Third Step)

- Makes viral nucleic acid available for transcription to permit multiplication to proceed
- Mechanism variably understood depending upon the virus
Uncoating of Influenza Virus

![Diagram of uncoating process](image)

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Steps in Viral Replication: Basic Strategies of Transcription and Translation (Fourth and Fifth Steps)

- (+) RNA → Proteins
- (-) RNA → (+) RNA → Proteins
- RNA → DNA → RNA → Proteins
- DNA → RNA → Proteins
Steps in Viral Replication: Assembly and Release (Sixth and Seventh Steps)

- Process involves bringing together newly formed genomic nucleic acid and structural proteins to form the nucleocapsid of the virus

- Nonenveloped viruses exhibit full maturation in the cytoplasm or nucleus with disintegration of cell

Steps in Viral Replication: Assembly and Release (Sixth and Seventh Steps)

- Many enveloped viruses exhibit full maturation as the virion exits the cell
  - Viral proteins are inserted into the host cell membrane
  - Nucleocapsids bind to these regions and bud into the extracellular space
  - Further cleavage and maturation of proteins may occur after viral extrusion
  - Cytolytic activity of these viruses varies
Influenza Virus

FIG. 2. Electron micrographs of purified influenza virus virions and virions budding from the surface of MDCK cells. A: Influenza A/3/30/72 virus negatively stained with HA decorated with 10 nm gold (<159,250). B: Influenza A/3/30/72 virus negatively stained with M, decorated with 10 nm gold (<159,250). C: Thin section of an influenza A/3/30/72 virus-infected MDCK cell with HA decorated with 10 nm gold (H&E, <40,000). D: Thin section of an influenza A/3/30/72 virus-infected MDCK cell with M, decorated with 10 nm gold (<40,000). Courtesy of George Lyster, Northwestern University, Evanston, IL.

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Retroviruses

FIG. 1. Ultrastructure of primate lentivirus. Electron microscopy of extracellular particles of HIV-1 (A) and SIVmac (B) reveals virions, about 110 nm in diameter, with a cone-shaped nucleoid surrounded by a lipid bilayer membrane, which contains envelope glycoprotein spikes (<100,000).

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Steps in Viral Replication: Assembly and Release
(Sixth and Seventh Steps)

- Herpesviruses (enveloped) assemble nucleocapsids in the nuclei of infected cells and mature at the inner lamella of the nuclear membrane
  - Virions accumulate in this space, in the ER and in vesicles
  - Virion release is associated with cytolysis

Herpes Simplex Virus

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Schematic of Replication Cycle of (+) RNA Single Strand Viruses Coding for One Sized RNA

Genomic RNA binds to ribosomes and is translated into polyprotein

Polyprotein is cleaved

Genomic RNA’s serve as templates for synthesis of complementary full length (-) RNA’s by viral polymerase

(-) strand RNA serves as template for (+) strand RNA’s; these serve to produce more polyprotein, more (-) strand RNA’s or become part of new virions

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Schematic of Replication Cycle of (+) RNA Single Strand Viruses Coding for Genomic and Subgenomic RNA’s

Genomic RNA binds to ribosomes but only a portion of 5’ end is translated into non-structural proteins

(-) strand RNA is synthesized. Different classes of (+) RNA’s are produced. One is translated into a polyprotein which is cleaved to form structural proteins. Another is full length and serves as genomic RNA for new virions

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Schematic of Nonsegmented (-) RNA Strand Virus Replication Cycle

Transcription of (-) strand occurs after entry and mediated by virion packaged transcriptase

(+)-strand RNA's produced; proteins synthesized

Full length (-)-strand RNA's produced and packaged into new virions

Transcription and translation take place entirely in cytoplasm

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Schematic of Segmented (-) RNA Strand Virus Replication Cycle

mRNA's are synthesized from each segment

Viral proteins are synthesized

(+) strand RNA's are synthesized and serve as templates for (-)-strand genomic RNA's

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Schematic of Herpesvirus Replication Cycle (DS DNA Virus Which Replicates in Nucleus)

Sequential, ordered rounds of mRNA and protein production regulate replication

Structural proteins produced during last cycle of replication

FIG. 10. Flow of events during the replication of herpesviruses (herpes simplex virus).

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HIV-1 Virion

Primary HIV Infection: Pathogenetic Steps

- Virus – dendritic cell interaction
  - Infection is typically with R5 (M-tropic) strains
  - Importance of DC-SIGN
- Delivery of virus to lymph nodes
- Active replication in lymphoid tissue
- High levels of viremia and dissemination
- Downregulation of virus replication by immune response
- Viral set point reached after approximately 6 months

PHI: Early Seeding of Lymphoid Tissue

Schacker T et al: J Infect Dis 2000;181:354-357
Primary HIV Infection:
Clinical Characteristics

- 50-90% of infections are symptomatic
- Symptoms generally occur 5-30 days after exposure
- Symptoms and signs
  - Fever, fatigue, myalgias, arthralgias, headache, nausea, vomiting, diarrhea
  - Adenopathy, pharyngitis, rash, weight loss, mucocutaneous ulcerations, aseptic meningitis, occas. oral/vaginal candidiasis
  - Leukopenia, thrombocytopenia, elevated liver enzymes
- Median duration of symptoms: 14 days

The Variable Course of HIV-1 Infection

Primary HIV Infection: Determinants of Outcome

- Severity of symptoms
- Viral strain
  - SI (X4) vs. NSI (R5) viruses
- Immune response
  - CTL response
  - Non-CTL CD8 responses
  - Humoral responses?
- Viral set point at 6-24 months post-infection
- Other host factors
  - Chemokine receptor and HLA genotype
- Gender and differences in viral diversity?
- Antiviral therapy
  - Near vs. long-term benefit?

Natural History of Untreated HIV-1 Infection

- CD4+ Cells
- Time in Years
- Early Opportunistic Infections
- Late Opportunistic Infections
Antiviral Agents for HIV

Mediated Fusion Inhibition

Modified from Furuta et al., Nature 387, 426-430 (1997)

Entry Inhibitors

Reverse transcriptase inhibitors Protease inhibitors

Mechanism of T20/T1249 Mediated Fusion Inhibition


gp120 Cell Membrane Fusion peptide HR1 HR2

Receptor Binding Δ Conformation

gp41 Virus Membrane Fusion Intermediate

Native Form

T20 T1249 Fusion Blockade

“Ensared” Transition State Intermediate

Membrane Fusion

Core Structure