Emerging Viruses

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CWA

Jan 23, 30, and Feb 6 2018
Part I Introduction to Virology

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Viral Lifecycle
Common Needs
Unique solutions
Transmission Routes
Classification
Methods of Visualizing
Virion Components
Capsid Structures
Viral Envelopes
Functions of Structural Proteins
Functions of Nonstructural Proteins
Part II Virus-Host Interactions, Vaccines and Inhibitors

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  Adaptive
Viral Countermeasures
Vaccines
  Vaccines Improve Life Expectancy
  Dependence upon Herd Immunity
  Passive Vaccines
  Active Vaccines
    Inactivated Virus
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  The Path of Drug Discovery
  The HIV experience
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Part III Emerging Viruses

Zoonoses and Why They Occur
Influenza, A Respiratory Virus
Ebola, A Blood-Borne Virus
Zika, A Mosquito Transmitted Virus
Emerging Viruses

Part I

Introduction to Virology
What is a Virus?

An infectious, obligate intracellular parasite comprising genetic material (DNA or RNA) surrounded by a protein coat and/or a membrane.
There is an underlying simplicity and order to viruses because of two simple facts

- All viral genomes are obligate molecular parasites that can only function after they replicate in a cell
- All viruses must make mRNA that can be translated by host ribosomes: they are all parasites of the host protein synthesis machinery
A virus is an organism with two phases. What would you do if you were a parasite that could only reproduce inside a cell?
A Virus is an Organism with Two Phases

Extracellular

Stable particle

Intracellular

Disassembly
Reproduction
New, stable particle
assembly and release
Common Needs

2 phases of viral lifecycle: extracellular and intracellular

- Find permissive cell
- Bind cell
- Penetrate membrane
- Uncoat
- Reprogram Cell for own replication
- Establish replication center
- Synthesize viral mRNA (transcription)
- Synthesize viral proteins (translation)
- Replicate genome
- Assemble and mature virion
- Release stable virion (lysis or budding)
Unique Solutions

ENTRY:
- Multiple receptors
- Multiple modes of entry
- Surface proteins
- Site of membrane penetration

- Uncoating
- Replication site
- Assembly
- Egress
- (Cell-to-cell) transmission
Sites of Viral Entry into the Host

V. Raconelli, MID 31
In humans, viruses cause a wide range of misery.
Virus classification

- Nature and sequence of nucleic acid in virion
- Symmetry of protein shell (capsid)
- Presence or absence of lipid membrane (envelope)
- Dimensions of virion & capsid
The seven classes of viral genomes

- dsDNA
- gapped dsDNA
- ssDNA
- dsRNA
- ss (+) RNA
- ss (-) RNA
- ss (+) RNA with DNA intermediate
Viruses are Nature’s Nanoparticles

- **Size range** –
  - most <0.2 μm; requires electron microscope
  (too small to be observed by traditional microscope)

1 μM = 1000 nM
The Tools of Viral Structural Biology

- X-ray Crystallography (must have crystals)
- But most viruses and their proteins are flexible and do not crystallize
- Electron microscopy (can see particles)

- CryoEM and Tomography (structures of flexible virions and proteins)
- NMR (structures of regions within protein)
The Structural Proteins
Capsid Functions

• Encases and protects genetic material
• Contains the budding function
• Maintains stable structure during transit to new host/cell
• Undergoes orderly disintegration during entry and travel to replication site
• For non-enveloped viruses, the capsid is the outer shell, contains the receptor binding site, and is the target for immune responses
Building virus particles: Symmetry is key

- Watson and Crick did more than discover DNA structure

- Their seminal contribution to virology:
  - Identical protein subunits are distributed with *helical symmetry* for rod-shaped viruses
  - *Platonic polyhedra symmetry* for round viruses  (cores have icosahedral symmetry)
Icosahedral Capsid Structures

- Characteristic size scale is 30-100 nm.
- Structures are known at “atomic resolution” - see Viper website (http://viperdb.scripps.edu/)
- Highly symmetric - think hard about what this implies about assembly!

(Baker et al.)
Symmetry and self-assembly

- Many capsid proteins can self assemble into virus-like particles (VLPs)
- The HBV and HPV vaccines are VLPs made in yeast

HPV 16 VLPs
Helical symmetry

Coat protein molecules engage in identical, equivalent interactions with one another and with the viral genome to allow construction of a large, stable structure from a single protein subunit.
Capsids can be covered by host membranes: enveoloped virions

- Envelope is a lipid bilayer derived from host cell
  - Viral genome does not encode lipid synthetic machinery
- Envelope acquired by budding of nucleocapsid through a cellular membrane
  - Can be any cell membrane, but is virus-specific
- Nucleocapsids inside the envelope may have helical or icosahedral symmetry
Viral Envelopes

- Membranes derived from Host Cell Membranes
- Contain Viral Proteins termed Surface Proteins
- Surface Proteins
  - bind target cells
  - define internalization pathways
  - **Fuse** viral and cellular membranes
  - Are targets of the immune response
- Because capsids usually contain budding information, enveloped viruses are promiscuous for surface protein incorporation
Viral envelope glycoproteins

- Integral membrane glycoproteins
- Ectodomain: attachment, antigenic sites, fusion
- Internal domain: assembly
- Oligomeric: spikes
Receptors Come in Many Forms
What we study: How **enveloped** viruses bind to, (enter), and fuse with cell membranes

Any thoughts on why we study this?
Virus particles are metastable

- Must protect the genome (stable)
- Must come apart on infection (unstable)
Virions are metastable

- Virus particles have not attained minimum free energy conformation
- Unfavorable energy barrier must be surmounted

- Energy put into virus particle during assembly (*spring loaded*)
- Potential energy used for disassembly if cell provides proper signal
Fusion is regulated

- Must not occur in the wrong location
- Neutral pH (plasma membrane):
  - Second protein receptor interaction
- Low pH fusion
  - Proteolytic cleavage activates the fusion protein for cleavage (class I)
  - Cleavage of a second protein (class II) activates the fusion protein
  - Endosome fusion receptor
INFLUENZA HA:
Conformational Changes During Fusion

Native: metastable structure
First change: Head-group separation
Activated: prehairpin
Post-fusion: 6-helix bundle
During fusion, fusion proteins convert from unique conformations to 6-helix bundles.
Common Viral Fusion Mechanism

Model is for a Class I fusion protein (flu HA, HIV Env, Ebola GP)
Viruses Exploit All Modes of Internalization

- Adenovirus (induces)
- Picornaviruses?, Influenza, Sendai?
- Influenza, VSV, SFV
- SV40, polyoma, MLV?

Replication

- DNA viruses replicate in the nucleus
- Most RNA viruses replicate in the cytoplasm
- Retroviruses reverse transcribe their RNA into DNA which then integrates into the host DNA for replication
- Viral polymerases make mRNAs
- Many viral proteins are synthesized as single polymer which must be cleaved into separate proteins for virus function
- Unique viral proteins include reverse transcriptases (RT), integrases (IN), polymerases (Pol), and proteases
All Virions Complete a Common Set of Assembly Reactions

Formation of individual structural units of the protein shell from one or several viral proteins

Assembly of the protein shell by appropriate, and sometimes variable, interactions among structural units

Selective packaging of the nucleic acid genome and other essential virion components

Acquisition of an envelope

Release from host cell

Maturation of the virus particles

Common to all viruses

Unique to enveloped viruses
Assembly is dependent on host cell machinery

- Cellular chaperones
- Transport systems
- Secretory pathway
- Nuclear import and export machinery
Sub-assemblies

- Ensure orderly formation of viral particles and virion subunits
- Formation of discrete intermediate structures
- Can’t proceed unless previous structure is formed: *quality control*
Acquisition of an envelope

- After assembly of internal structures (most enveloped viruses)

I. Nucleocapsid
   - Envelope glycoproteins and capsid essential for budding - alphaviruses

II. Matrix
   - Internal matrix or capsid proteins drive budding - retroviruses

III. Envelope proteins drive budding - influenza virus, coronavirus

IV. Matrix proteins drive budding, but additional components (glycoproteins, RNP) needed for efficiency or accuracy

Membrane-associated particle
Membrane tethers
Other virion components

- Enzymes
  - polymerases, integrases, associated proteins
  - proteases
  - poly(A) polymerase
  - capping enzymes
  - topoisomerase
- Activators, mRNA degradation, required for efficient infection, mRNAs
- Cellular components - histones, tRNAs, myristate, lipid, cyclophilin A, and many more
All Steps in the Viral Life Cycle are Targets for Intervention

The HIV Life Cycle
Questions?
Emerging Viruses

Part Ila
Virus-Host Interactions
The Two Arms of the Immune Response: Innate vs Adaptive Immunity

<table>
<thead>
<tr>
<th>Innate</th>
<th>Adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Primitive: In all multicellular organisms</td>
<td>• Only in vertebrates</td>
</tr>
<tr>
<td>• Directed toward classes of molecules</td>
<td>• Directed toward specific epitopes on molecules</td>
</tr>
<tr>
<td>• Effectors broadly reactive</td>
<td>• Effectors highly specific</td>
</tr>
<tr>
<td>• No anamnestic response</td>
<td>• Memory persists</td>
</tr>
<tr>
<td>• Effectors = epithelial cells, phagocytes,</td>
<td>• Effectors = lymphocytes, Antigen Presenting</td>
</tr>
<tr>
<td>endothelial cells, fibroblasts</td>
<td>Cells (APCs)</td>
</tr>
</tbody>
</table>

Interferons (IFN) are major components of innate immune response

- Activated within minutes to hours after infection
- Cytokines, sentinel cells (dendritic cells, macrophages, NK cells), complement
- Can inform adaptive response when infection reaches dangerous threshold
B-Cells

Make Antibodies
Make Cytokines to Stimulate Innate Immunity
Steady-state Immune Readiness
Regulate Immune Response
Stimulate T-cells
Three broad categories of T cells

**Helper T cells** augment the immune response by recognizing the presence of a foreign antigen and then stimulating antibody production and producing cytokines that “turn on” or activate other T cells.

**Regulatory T cells** function in an opposite manner: they dampen or turn off the immune response.

**Cytotoxic or “killer” T cells** directly attack and destroy cells bearing antigenic material.
# Three classes of cytokines

<table>
<thead>
<tr>
<th>Group</th>
<th>Some members</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proinflammatory</td>
<td>IL-1, Tnf, IL-6, IL-12</td>
<td>Promote leukocyte activation</td>
</tr>
<tr>
<td>Antinflammatory</td>
<td>IL-10, IL-4, Tgf-β</td>
<td>Suppress PICs</td>
</tr>
<tr>
<td>Chemokines</td>
<td>IL-8</td>
<td>Recruit immune cells</td>
</tr>
</tbody>
</table>

Initially function locally in antiviral defense. In larger quantities, enter circulation, have global effects (sleepiness, lethargy, muscle pain, no appetite, nausea).

*A localized viral infection produces global effects*
Inflammation usually stimulates potent immune responses

- Cytopathic viruses cause inflammation because they promote cell and tissue damage
  - Activate the innate response
- Consequently cytopathic viral genomes encode proteins that modulate this immune response
  - Adenoviruses, herpesviruses, poxviruses
Some viruses do not stimulate inflammation

- Typically non-cytopathic viruses
  - Cells are not damaged, no apoptosis/necrosis
  - Low or ineffective innate immune response
  - Do not effectively activate adaptive immune response

- Non-cytopathic viruses have dramatically different interactions with the host immune system
  - Persistent infections: rarely or inefficiently cleared
Viral Interference with Early B-cell Activation
Viral Interference with Antibody Production and High Affinity Maturation

M. Kuka and M. Iannacone, doi: 10.1038/nr, 2017.133i
### Table 1 | Viral subversion of B cell responses

<table>
<thead>
<tr>
<th>B cell activation step</th>
<th>Virus</th>
<th>Effect on B cell function</th>
<th>Molecular mechanism</th>
<th>Effect on Ab production</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag encounter and early B cell activation</td>
<td>Influenza virus</td>
<td>Ag-specific B cell infection and killing</td>
<td>BCR–haemagglutinin interaction</td>
<td>Suppression of primary Ab responses</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Vaccinia virus and influenza virus</td>
<td>Disruption of the lining of SCS macrophages</td>
<td>TLR9-mediated and MyD88-mediated signalling</td>
<td>Suppression of secondary Ab responses</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>HIV</td>
<td>Inhibition of B cell proliferation</td>
<td>gp120–α4β7 integrin interaction; induction of TGFβ and FcRL4</td>
<td>ND</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Measles virus</td>
<td>ND</td>
<td>ND</td>
<td>Suppression of Ab secretion</td>
<td>54, 55</td>
</tr>
<tr>
<td></td>
<td>CMV</td>
<td>Inhibition of BCR signalling</td>
<td>LMP2A sequesters LYN and SYK kinases</td>
<td>ND</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>EBV</td>
<td>Inhibition of BCR signalling and polyclonal expansion</td>
<td>E2–CD81 interaction leads to downregulation of CD21 and upregulation of anti-apoptotic genes</td>
<td>Low levels of polyclonal Ab</td>
<td>62, 63</td>
</tr>
<tr>
<td></td>
<td>HCV</td>
<td>Inhibition of BCR signalling</td>
<td>Sustained type I interferon and CCL2 production</td>
<td>Lack of early neutralizing Ab</td>
<td>47</td>
</tr>
</tbody>
</table>

| B cell relocalization | LCMV           | Recruitment of inflammatory monocytes and B cell apoptosis      | Hypersecretion of total IgG                               | 71                                           |
| Interaction with T helper cells | HIV | Polyclonal hyper-gammaglobulinaemia                             | Hypersecretion of nonspecific Ab                          | 70, 73, 75                                   |
|                         | LCMV           | Non-specific B cells present viral Ag                           | Hypersecretion of nonspecific Ab                          | 70, 72, 73                                   |
|                         | MHV-68         | ND                                                              | Hypersecretion of nonspecific Ab                          | 69                                           |
|                         | Adenovirus     | ND                                                              | Non-specific Ab responses                                  | 72                                           |
|                         | LDV            | ND                                                              | Hypersecretion of nonspecific Ab                          | 70, 72, 73                                   |
|                         | LCMV           | Decreased help from T<sub>HM</sub> cells                        | NK-mediated suppression of T<sub>HM</sub> cells           | Lack of specific Ab                          | 76, 77 |
|                         | Pichinde virus | ND                                                              | ND                                                       | 77                                           |

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<tr>
<td>Differentiation into plasma cells</td>
<td>LCMV</td>
<td>Differentiation into short-lived plasma cells and B cell apoptosis</td>
<td>Sustained type I interferon production, induction of TNF and IL-10</td>
<td>Lack of early neutralizing Ab</td>
<td>79</td>
</tr>
<tr>
<td>Germinal centres</td>
<td>HIV</td>
<td>Inhibition of CD40L-dependent immunoglobulin class switching</td>
<td>Nef-mediated induction of IκBα and SOCS proteins</td>
<td>Nonspecific Ab responses</td>
<td>85, 86</td>
</tr>
<tr>
<td></td>
<td>HIV</td>
<td>Polyclonal hypermutation</td>
<td>gp120 binding to MCLR induces AID expression</td>
<td>Production of class-switched Ab</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>HCV</td>
<td>E2–CD81 interaction induces AID expression and hypermutation</td>
<td></td>
<td>Production of low-affinity antibodies</td>
<td>88–90</td>
</tr>
<tr>
<td></td>
<td>BTV</td>
<td>FDC infection and killing</td>
<td>ND</td>
<td>Lack of neutralizing Ab</td>
<td>91</td>
</tr>
<tr>
<td>CD8+ T cell response</td>
<td>Measles virus</td>
<td>CD8+ T cell-mediated B cell killing</td>
<td>ND</td>
<td>Suppression of Ab secretion</td>
<td>92</td>
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<tr>
<td></td>
<td>LCMV</td>
<td></td>
<td>Sustained type I interferon production</td>
<td>Lack of early neutralizing Ab</td>
<td>74, 94</td>
</tr>
<tr>
<td></td>
<td>HBV</td>
<td>B cells present viral Ag on MHC class I</td>
<td>ND</td>
<td></td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>LCMV</td>
<td>Generalized immunopathology and disruption of follicular architecture</td>
<td>ND</td>
<td>Lack of early neutralizing Ab</td>
<td>99–102</td>
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Questions?