Viral Evasion Strategies

Lecture 14
Virology W3310/4310
Spring 2012
“Don’t look back, something might be gaining on you!”

Satchel Paige
Host vs. Virus

• What one does to the other?

• Evolution of strategies to evade innate and adaptive cell responses to infection
  - goal: survival, reproduction and release
Strategies for Evasion

- Overwhelm the host

- Enter parenterally
  - Anyway other than the gut
  - Why?

- Disarm host defenses
Acute Infections

When to treat?

Symptoms

Virus growth

Establishment of infection

Innate defenses

Induction of adaptive response

Adaptive response

Memory

Threshold level of virus required to activate adaptive immune response

Entry of virus

Duration of infection

Virus cleared
Virus Offense Meets Host Defense

[Diagram showing the interaction between pathogen and host defense systems, including pattern recognition receptors, innate response, and adaptive immune response stages.]
Evasion

• Disarm innate immunity

• Regulate MHC molecules
  - responsible for antigen presentation

• Interfere with CTL and NK cells

• Alter antigen presentation

• Go and hide
  More on this later
Host Defenses

• Immune system, recognizes signal, amplifies signal and controls invader

• Innate immunity, responds to everything

• Intranuclear modulatory molecules

• Interferons induce an antiviral state

• PKR

• Complement punches holes in membranes

• NK and CTLs

• Macrophages and neutrophils
Adaptive Immunity

• A memory response
• Activated in response to the innate immune system
• Results in clonal expansion of B and T cells
  - requires Th1 and Th2 cells
• Generation of Cytotoxic T cells and antibody production
Inflammatory Response

- Occurs in response to necrosis
- Results in release of cytokines and chemokines
- Recruits neutrophils and macrophages to site of damage
Cytokines

- Primary output of innate immune response
- Rapid induction in response to infection
- Control inflammation
- Induce antiviral state, IFNs
- Regulate immune system
Viral Response

• Virokines, it looks like, smells like, so don’t step in it
  - these mimetics bind and sequester host receptor molecules

• Viroceptors
  - soluble cytokine receptors
  - divert cytokines from initiating response

• Sabotage innate and adaptive defense without affecting growth in cell culture
Products that Counter IFN

• Why?
  - Without IFN host has a reduced ability to contain viral infections

• dsRNA binding proteins
  - NS1 from Influenza
  - E3h from Poxviruses
  - US11 from HSV

• Ad VA-RNA
  - A dsRNA decoy that binds PKR
# Modulators of the IFN Response

<table>
<thead>
<tr>
<th>Type of modulation</th>
<th>Representative viruses</th>
<th>Viral protein, if known</th>
<th>Mechanism of action</th>
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</thead>
<tbody>
<tr>
<td>Inhibition of IFN synthesis</td>
<td>Epstein-Barr virus</td>
<td>BcRF1</td>
<td>IL-10 homolog, inhibits production of IFN-γ</td>
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<td></td>
<td>Vaccinia virus</td>
<td>A18R</td>
<td>Regulates dsRNA production</td>
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<td>Foot-and-mouth disease virus</td>
<td>L</td>
<td>Host protein synthesis block</td>
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<tr>
<td>IFN receptor decoys</td>
<td>Vaccinia virus</td>
<td>B18R</td>
<td>Soluble IFN-α/β decoy receptor</td>
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<tr>
<td>Inhibition of IFN signaling</td>
<td>Adenovirus</td>
<td>E1A</td>
<td>Decreases quantity of Stat1 and P48; blocks Isgf3 formation; interferes with Stat1 and CBP/P300 interactions</td>
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<tr>
<td></td>
<td>Vaccinia virus</td>
<td>VH1</td>
<td>Viral phosphatase reverses Stat1 activation</td>
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<td></td>
<td>Human papillomavirus 16</td>
<td>E7</td>
<td>Binds p48</td>
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<tr>
<td></td>
<td>Hepatitis C virus</td>
<td>NS5a</td>
<td>Blocks formation of Isgf3 and Stat dimers</td>
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<td></td>
<td>Nipah virus</td>
<td>V protein</td>
<td>Prevents Stat1 and Stat2 activation and nuclear accumulation</td>
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<tr>
<td>Block function of IFN-induced proteins</td>
<td>Adenovirus</td>
<td>VAI RNA</td>
<td>Binds dsRNA, blocks Pkr</td>
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<tr>
<td></td>
<td>Herpes simplex virus type 1</td>
<td>Us11</td>
<td>Blocks Pkr: activation</td>
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<td>γ34.5</td>
<td>Redirects protein phosphatase 1α to dephosphorylate eIF2α; reverses Pkr action</td>
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<td>Vaccinia virus</td>
<td>E3L</td>
<td>Binds dsRNA and blocks Pkr</td>
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<td></td>
<td>K3L</td>
<td>Pkr pseudosubstrate, decoy</td>
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<tr>
<td></td>
<td>Human immunodeficiency virus type 1</td>
<td>Tar RNA</td>
<td>Blocks activation of Pkr</td>
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<td>Tat</td>
<td>Pkr decoy</td>
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<td></td>
<td>Hepatitis B virus</td>
<td>Capsid protein</td>
<td>Inhibits MxA</td>
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<td>Influenza virus</td>
<td>NS1</td>
<td>Binds dsRNA and Pkr</td>
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<td></td>
<td></td>
<td>NS1</td>
<td>Blocks action of ISG15</td>
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<tr>
<td></td>
<td>Reovirus</td>
<td>σ3</td>
<td>Binds dsRNA, inhibits Pkr and 2' 5' oligo (A) synthase</td>
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Regulators of ISGs

- ND10s are composed of host proteins that repress virus replication
  - innate nuclear defense
  - epigenetic regulation of virus replication
  - PML, an important ND10 constituent

- HSV ICP0 targets PML for proteolysis
Dissolution of PML

HSV

ICP0

PML

merge

VZV

ORF61p

PML

merge
Translational Regulation

• Viruses modify host to favor synthesis of their own proteins

• IFNs establish an anti-viral state

• Induction of Protein Kinase R and other eIF2α kinases
  - inhibit translation
  - consequences for virus replication
Innate Defense Targets
PKR

- Activated by binding dsRNA
- Autophosphorylates at S_{51}
- Phosphorylates eIF2α
  - forms a very tight ternary complex with GDP- eIF2B
  - blocks recycling
  - translation is arrested
How Viruses Counteract Pkr

- Virus proteins have evolved to thwart host anti-virus defenses
- Herpes simplex virus US11 blocks Pkr activation
- Adenovirus VA RNAs bind tightly to Pkr
  - dsRNA decoy
- HPV E6 and HSV γ34.5 dephosphorylate eIF2α
  - γ34.5 interacts with PPIa redirecting it to eIF2α
Phosphorylation of eIF2α

US11 a protein
Ad VA RNAs
HPV E6
HSV γ34.5
Viral Modulators of Interferon

- Inhibit IFN synthesis
- IFN Receptor decoys
- Inhibition of IFN signaling
- Block function of Interferon Stimulated Genes
Autophagy, a **catabolic** process involving degradation of a cell's own components through the **lysosomal** machinery

![Diagram of autophagy process](image)
Stimulation and Inhibition of Autophagosome Formation

Mock  HCMV  UV-HCMV

4 h

GFP-LC3  GFP-LC3  GFP-LC3

pp65  pp65  pp65

8 h

GFP-LC3  GFP-LC3  GFP-LC3

pp65  pp65  pp65

24 h

GFP-LC3  GFP-LC3  GFP-LC3

pp65  pp65  pp65

LC3 an autophagy marker

TRS1 an Inhibitor

pp65 HCMV protein

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Apoptosis

- Host escape mechanism leading to cell death and inflammation by cysteine proteases (Caspases)
  - induction of cytokines
  - infected cells release proteins that are subsequently presented by MHCII
  - activation of CTLs
Apoptosis

• **Catabolic** process involving degradation of a cell's own components through the lysosomal machinery

• Host controls induction and suppression

• Antiapoptotic:
  BCL-2 family members block mitochondrial translocation

• Proapoptotic:
  BAX and BAD cause induction of a caspase cascade by release of mitochondrial cytochrome C
Intrinsic Death Receptor Pathway

Activators

Oligomerization

Inhibitors
Extrinsic Death Receptor Pathway

Ligands
Receptors
Activation of Caspase 8
Characteristics of Apoptosis

- Cell organelles are dismantled
- Vesicle formation and membrane blebbing
- DNA is cleaved
- Phosphatidylserine, annexin appear on cell surfaces
Apoptosis

- Perturb the cell cycle and apoptosis is activated - cell falls apart and virus fails to complete replication cycle

- Block it and virus can complete replication
Characteristics of Apoptosis

- Membrane blebbing and apoptotic body formation
Characteristics of Apoptosis

• HSV ICP27 prevents DNA fragmentation
Why Block Apoptosis?

• Why are cells induced after infection?
  - activation of quiescent cell machinery
  - checkpoint controls respond

• Virus responds to complete replication
  - failure leads to decreased yields

• Inhibit release of virus Antigens
  - eliminate T cell activation
  - evade immune response
Apoptotic Pathways and Viruses
How to Block It

• HCMV transcribes a noncoding RNA (β2.7) that binds a mitochondrial protein that triggers apoptosis

• AD E1B binds BAX preventing caspase activation (intrinsic)

• AD E3 blocks Fas (Death Receptor) - induced apoptosis (extrinsic)
HIV Evades the Rig I Innate Immune Response
HIV Sequesters RIG-I to Lysosomes Where It Is Degraded

- GFP-Protease
- RIG-I
- LAMP-1
- Merge

GFP
- RIG-I Cytoplasmic

GFP-Protease
- RIG-I Perinuclear

GFP-Protease + Saquinavir
- RIG-I Perinuclear
Apobec

- A protein family whose function is to edit RNA
  - an ISG
  - deaminates C → U

- Intrinsic antiviral

- Blocks replication of HIV, HBV and Measles

- Incorporated into HIV virus particles
  - Is the host a step ahead here?
How Apobec Inhibits HIV Replication

- C’s in - strand DNA are deaminated
- C→U transitions result in GC→AT pairs
  - TGG (W) codons become TAA (Stop)
- U containing DNA is attacked by U-DNA glycosidase
  - generates abasic site and becomes a target for endonucleases
How HIV Survives Apobec

- HIV encodes Vif which is incorporated in the virion
- Vif interacts in a species specific manner to bind Apobec and target it to the proteasome
Humoral & Cell Immunity

Antigens

Foreign proteins  Viruses  Bacteria  Parasites  Fungi

Humoral response + Cell-mediated response

Vertebrate body

B cells activated

B cell + Virus particles (antigen)

Plasma cells secrete antibodies

Antigen eliminated

Bone marrow

Naïve T cells

Thymus

CD4

Th precursor

CD8

CTL precursor

Lymph node

Burst of Th1 cytokines

Th cell

CD4

Burst of Th2 cytokines

CTL

Killing of infected self cells

Infected self cell

MHC II

MHC I

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Effects of Stimulating Cell Division

- Clonal expansion
  - increases # of cell responders

- T cells kill cells bearing foreign peptide or protein
  - CD4$^+$ Th recognize MHC II bound peptides
  - CD8$^+$ Tc recognize MHC I bound peptides

- In response to cytokines from Th1 cells CD8$^+$ become mature CTLs

- In response to cytokines from Th2 cells B cell synthesizes Ab (becomes a plasma cell)

- B cell can also become a memory cell
Adaptive Immunity

- Humoral

- Cell-mediated, recognition mediated by:
  - membrane bound Ab on B cells and or TCR
  - Ag presented by MHC I on all cells
  - Ag presented by MHC II on macrophages or dendritic cells
BCR and TCR

- Both bind antigen
- B cells as linear epitopes
- T cells as peptides
- Binding releases cytokines
  - stimulates cell division
T Cell Function

- CD4⁺ Th cells differentiate into Th1 and Th2 cells

**Cross Regulation**

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<th>Th2 response</th>
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<td>Enhance</td>
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</tr>
<tr>
<td>Suppress</td>
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- IL-2, IL-12, IFN-γ enhance Th1 response
- IL-4, IL-10 enhance Th2 response
- IL-12, IL-10 suppress Th1 response
- IL-5, IFN-γ suppress Th2 response

Cell-mediated immunity dominates proinflammatory response

Antibody response dominates

**Enhance**
- IL-2
- IL-12
- IFN-γ

**Suppress**
- IL-4
- IL-10

**Enhance**
- IL-4
- IL-5
- IL-6
- IL-10

**Suppress**
- IL-12
Two signal systems, MHC and accessory molecules and their ligands - lead to T cell activation
**Immune Modulation**

- Dendritic cells are often compromised by virus infection resulting in suppression of the immune response by:
  - interference with recruitment
  - impairment of Ag uptake or processing
  - interference with maturation
  - inability to migrate to lymphoid tissue
  - failure to activate T cells
Remediation of T Cell Response

- EBV encodes an IL 10 homologue that suppresses the Th1 response
  - thus sparing infected B cells
- KSHV elaborates a horde of virokines (cytokine mimetics) that provide a refuge from immune surveillance
Natural Killer Cells

- No antigen receptors
- Secrete cytokines
- Kill cells lacking MHC I on the surface by recognizing missing self
- ADCC, NKs bind to IgG coated cells, release perforins and granzymes triggering apoptosis
Virus Modulation of NK Cells

- MHC I homologs
- Regulators of MHC I
- Release of virus-encoded cytokine-binding proteins (Viroceptors) block engagement of activating receptor
- Antagonist of activating receptor
- Infection of NK cell can lead to disruption of function or cell death
Virus Modulation of NK Cells
Virus Modulation of NK Cells

• HCV E2 protein binds CD81 on NK cells to block activation
  - don’t recognize infected cells

• HIV nef affects cell surface expression of some MHC I molecules but not HLA-E (cell is still protected)

• Poxviruses express proteins that bind IL-12 to inhibit IFN-γ production by NKs
Exogenous Antigen Presentation
MHC I Presentation

- MHC I
- β2μ
- Tap 1
- Tap 2
- Calnexin
- Proteasome
- Lumen of ER
- Peptide in binding groove
- α chain
- β2μ
- Cytoplasm
- Protein
- Ub-dependent proteolysis
- Transport vesicles
- Golgi
- cis
- medial
- trans
- Presenting cell
- T-cell receptor
- CD8
- T cell
- Tuesday, March 6, 2012
• Viral proteins interfere with MHC I mediated antigen presentation

• Just how many targets are there?
Intercession by HSV-1 ICP47

- Prevents binding of peptides to TAP
  ICP 47 retained in the ER
Intercession by Ad E19

- Blocks MHC I -Tapasin interaction excluding it from the peptide loading complex
• A 23k Da ER-restricted glycoprotein with luminal and cytoplasmic domains that inhibits ATP binding to TAP thus preventing peptide loading
Intercession by EBV BNLF2a

- Prevents peptide and ATP binding to TAP like ICP47 and US6
• A glycoprotein that interacts with Tapasin resulting in MHC I heavy chain degradation
• Targets peptide-loading complex-incorporated MHC I for degradation by ubiquitination, interacts with both TAP and Tapasin
Cytomegaloviruses a Paradigm for Interference with MHC

- UL83 tegument protein inhibits proteasome processing by P*
- US6 inhibits MHC I translocation to the ER
- US3 inhibits MHC I transport across ER
- US2 & 11 force MHC I to cytoplasm
- UL18 is a MHC I mimetic thought to downregulate NK and CTLs
MHC Intercession Summary

• Viruses have to cope with their hosts
• For the host to succeed viral antigens must be presented on the cell surface
• Viruses have identified many points of intercession and defined how MHC I presents antigen
Immune Modulation Strategies

- Secreted Modulators
- Modulators on infected cell surface
- Stealth
- Antigenic hypervariability
- Bypass or kill lymphocytes
- Block adaptive immune response
- Inhibit complement
- Modulate apoptosis
- Modulate autophagy
- Interfere with pattern recognition receptors