Intrinsic and innate defenses

Lecture 13
Biology W3310/4310
Virology
Spring 2014

The trouble with facts is that there are so many of them
—Anonymous
Physical and chemical defenses
Skin, mucus, tears, low pH, surface cleansing
Host defenses

- Intrinsic
  - *Always present* in the uninfected cell
  - Apoptosis, autophagy, RNA silencing, antiviral proteins

- Innate immune system: *Induced* by infection

- Adaptive immune system: *Tailored* to pathogen
RNA interference

Plant & invertebrate cells
Mammals - present or not needed?
APOBEC3 and HIV-1
Autophagy

Phagophore → Autophagosome → Lysosome → Degradation
Apoptosis

Normal cell → Apoptosis begins → Apoptotic bodies → Macrophage
## Viral regulators of apoptosis

<table>
<thead>
<tr>
<th>Cellular target</th>
<th>Virus</th>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2</td>
<td>Adenovirus</td>
<td>E1B 19K</td>
<td>Bcl-2 homolog</td>
</tr>
<tr>
<td>Caspases</td>
<td>Adenovirus</td>
<td>14.7K</td>
<td>Inactivates caspase 8</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>Hepatitis B virus</td>
<td>pX</td>
<td>Blocks p53 mediated apoptosis</td>
</tr>
<tr>
<td></td>
<td>Human papilloma virus</td>
<td>E6</td>
<td>Targets p53 degradation</td>
</tr>
<tr>
<td></td>
<td>Simian virus 40</td>
<td>Large T</td>
<td>Binds and inactivates p53</td>
</tr>
<tr>
<td>Fas/TNF receptors</td>
<td>Adenovirus</td>
<td>E3 10.4/14.5K</td>
<td>Internalizes Fas</td>
</tr>
<tr>
<td></td>
<td>Cowpox</td>
<td>CrmB</td>
<td>Neutralizes Tnf and LT-α</td>
</tr>
<tr>
<td></td>
<td>Myxoma virus</td>
<td>MT-2</td>
<td>Secreted Tnf receptor homolog</td>
</tr>
<tr>
<td>vFLIPs; DED box-containing proteins</td>
<td>Human herpesvirus 8</td>
<td>K13</td>
<td>Blocks activation of caspases by death receptors</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Molluscum contagiosum virus</td>
<td>MC066L</td>
<td>Inhibits UV- and peroxide-induced apoptosis; homologous to human glutathione peroxidase</td>
</tr>
<tr>
<td>Transcription</td>
<td>Human cytomegalovirus</td>
<td>IE1, IE2</td>
<td>Inhibits Tnf-α but not UV-induced apoptosis</td>
</tr>
</tbody>
</table>

HCMV produces a 2.7 kb noncoding RNA that binds a mitochondrial protein needed for apoptosis.
Intrinsic defenses are always present. Which of the following are included?

1. Antibodies
2. T cells
3. Autophagy
4. All of the above
5. None of the above
Innate immune system

- 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
Pathogen not recognized

No innate response

Pattern recognition receptors

Cytokines

Dendritic cells

Complement cascade

Innate response

NK cells

Dendritic cells

Cytokines

Adaptive immune response

Evaluate pathogen structure
Fine-tune recognition

• Clonal expansion of B and T cells
• Memory
How does the innate system recognize microbes and not self?


• 1996: Toll found to have a role in immunity of fly to fungal infections

• 1997: Toll-like receptors identified in mammals
Cytoplasmic helicases
Sensing DNA

Microbes (viruses, bacteria, protozoa)

V. cholerae

Dictyostelium?

Sources of DNA

IFI16

DDX41

Bacteria

c-di-GMP

c-di-AMP

cGAS

cGAMP

STING

ATP + GTP

IKK

TBK1

NF-κB

IRF3

Immune and inflammatory genes

Hormones

GPCRs

Adenylation cyclase

cAMP

PKA

Physiological response

Many substrates

http://www.sciencemag.org/content/339/6121/763
Viral modulators of sensing cytoplasmic helicases

TLRs
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Which of the following allow the innate immune system to distinguish microbes from self?

1. Cytoplasmic helicases and TLRs
2. Antibodies
3. Apoptosis
4. Apobec
5. All of the above
Interferons

• 1957: Issacs & Lindenmann; chicken cells exposed to non-infectious influenza virus produce substance that “interfered” with infection of other cells

• Produced by virus-infected cells and uninfected sentinel cells in response to products released from cells (e.g. viral nucleic acid)
# Interferons

<table>
<thead>
<tr>
<th>Interferon&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Producer Cells</th>
<th>Inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α</td>
<td>Most if not all nucleated cells</td>
<td>Viral infection</td>
</tr>
<tr>
<td>IFN-β</td>
<td>Most if not all nucleated cells</td>
<td>Viral infection</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Tcells, NK cells</td>
<td>T-cell receptor activation, IL-2, IL-12</td>
</tr>
</tbody>
</table>

IFN-λ (type III) mainly produced by epithelial cells

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<sup>a</sup> Interferons are proteins produced by infected cells in response to viral infection. They are produced by various cells depending on the type of interferon.
• Production of IFNα/β is rapid: within hours of infection, declines by 10 h

• IFN binding to IFN receptors leads to synthesis of >1000 cell proteins (ISGs, IFN stimulated genes)

• Mechanisms of most ISGs not known
Interferon-induced proteins

RNase L, 2’-5’-oligo(A) synthetase

Viral dsRNA → 2'-5' OAS (Inactive) → 2'-5' OAS (Active) → ATP → 2'-5' oligo(A) → RNase L (Inactive) → RNase L (Active) → Viral mRNA → Viral mRNA Cleavage Products
Identification of ISGs that inhibit poliovirus

Replication = % GFP⁺ cells in RFP⁺ population normalized to Fluc control (PV)
The IFN system is dangerous

• IFN induces the expression of many deleterious gene products - most of our cells have receptors

• Large quantities of IFN have dramatic physiological consequences: fever, chills, nausea, malaise

• Every viral infection results in IFN production, one reason why ‘flu-like’ symptoms are so common
Viral modulators of IFN

Virology, Wolters Kluwer
How do interferons (IFNs) limit viral replication?

1. IFNs directly inhibit viral translation
2. IFNs induce TLRs
3. IFNs induce ISGs
4. IFNs damage cells
5. None of the above
Sentinel cells

- Dendritic cells, macrophages, natural killer (NK) cells
- They patrol all our tissues looking for signs of change
Sentinel cells

- Dendritic cells: first described as Langerhans cells by Paul Langerhans (late 19th century)
- Called dendritic cells in 1973 by Steinman & Cohn

TWiV 157: Better innate than never
DCs

Immunologically mature dendritic cells

Virus, virus protein
Inflammatory cytokines
Dead and dying cells

Toll-like receptor
Endosome
Cytokine receptor
MHC class II

IFN

Maturation
Nf-κB activation
Migration to lymph node

Mature dendritic cell

MHC class II viral peptides

Tcr

CD28

Contact

Cytokines IL-12, Tnf-α, IL-1β

Naive T cell

Activated T cells

Helper T cells

Principles of Virology, ASM Press
Viral modulation of NK cells

1 - Mimetic

2 - Downregulation & substitution

3 - Block activating proteins

4 - Inhibition of NK-stimulating cytokines or antagonist of activating chemokines

5 - Block receptor or kill cell
Classical cascade begins when a pathogen is detected by $C1q$ (there are two other detector systems)

Yes, there are viral modulators
Four functions of complement

- **Cytolysis** - make holes in infected cells if they have bound antibody or have unusual proteins on their surface
- Activation of *inflammation* - cytokines
- **Opsonization** - coat virus particles to facilitate uptake by macrophages
- **Solubilization** of immune complexes - break up antibody-antigen complexes that can damage organs
What roles do dendritic cells (DCs) play in viral defense?

1. DCs engulf and destroy virus particles
2. DCs sense infected cells and produce IFNs
3. DCs only instruct the adaptive response
4. DCs lyse virus-infected cells
5. All of the above
Infection leads to the inflammatory response

- Infected cells produce cytokines
- Redness; pain; heat; swelling, the four classic signs of inflammation (rubor, dolor, calor, tumor, originally recorded by the Roman encyclopedist Celsus in the first century AD)
- Result from increased blood flow, increased capillary permeability, influx of phagocytic cells, tissue damage
Three classes of cytokines

<table>
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<tr>
<th>Functional group</th>
<th>Selected members</th>
<th>Activity</th>
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<tbody>
<tr>
<td>Proinflammatory</td>
<td>IL-1, Tnf, IL-6, IL-12</td>
<td>Promote leukocyte activation</td>
</tr>
<tr>
<td>Antiinflammatory</td>
<td>IL-10, IL-4, Tgf-β&lt;sub&gt;b&lt;/sub&gt;</td>
<td>Suppress activity of proinflammatory cytokines; return system to basal “circulate and wait” state</td>
</tr>
<tr>
<td>Chemokines</td>
<td>IL-8</td>
<td>Recruit immune cells during early stages of immune response</td>
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Initially function locally in antiviral defense
In larger quantities, enter circulation, have global effects (sleepiness, lethargy, muscle pain, no appetite, nausea)
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 effectively activate the adaptive immune response

- Do not stimulate inflammatory response
- Typically non-cytopathic viruses
  - Cells are not damaged, no apoptosis/necrosis
  - Low or ineffective innate immune response
- Non-cytopathic viruses have dramatically different interactions with the host immune system
  - Persistent infections: rarely or inefficiently cleared
The lesson

- The classic inflammatory response (heat, swelling, redness, pain) reflects the communication of innate and adaptive immune defense

  - No inflammatory response, ineffective adaptive response

- One reason for using inflammation-stimulating adjuvants for noninfectious vaccines
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What is inflammation?

1. Heat, swelling, redness, pain
2. Increased blood flow, increased capillary permeability, influx of phagocytic cells, tissue damage
3. A consequence of cytokine production
4. Mainly caused by cytopathic viruses
5. All of the above
Cytokine countermeasures

**Ways to interfere with cytokine function**

**Interrupt cytokine production**
- Interfere with cytokine and chemokine synthesis
- Inhibit the generation of functional cytokines

**Interfere with cytokine action**
- Encode homologs of cytokines and cytokine receptors
  - Type I interferon (IFN) homolog: VV (B18R)
  - IFN-γ homolog: VV (B8R)
  - Interleukin (IL)-6: KSHV (K2)
  - IL–8 homolog: HCMV (UL146, 147)
  - IL–10 homolog: EBV (BCRF1), HCMV (UL111A)
- Generate soluble cytokine receptors to neutralize cytokines
  - IFN-γ receptor: myxoma virus (MT–7)
  - IL-1βR: VV WR (B15R)
  - TNFR homolog: orthopoxvirus (CrmB, CrmD)

**Interfere with cytokine effector function**
- Alter cytokine signaling pathway
Viral countermeasures

All viruses must encode at least one regulator of intrinsic/innate defenses