Lecture 9

Innate Immunity

Guest Lecture:
Joel Wertheim
Outline

• General Function
• Main Cellular Players
• Toll-like Receptors
• Interferon
• Complement System (3)

• APOBEC3G
<table>
<thead>
<tr>
<th>Receptor characteristic</th>
<th>Innate immunity</th>
<th>Adaptive immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity inherited in the genome</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Expressed by all cells of a particular type (e.g., macrophages)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Triggers immediate response</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Recognizes broad classes of pathogen</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Interacts with a range of molecular structures of a given type</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Encoded in multiple gene segments</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Requires gene rearrangement</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Clonal distribution</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Able to discriminate between even closely related molecular structures</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Figure 2-10 Immunobiology, 6/e. (© Garland Science 2005)
Innate vs. Adaptive
Timeframe

Figure 2-1 Immunobiology, 6/e. (© Garland Science 2005)
Importance of Innate Immunity

- Lacking adaptive immunity results in a slight increase in pathogen load and a substantial increase in the length of infection.
- Lacking innate immunity results in uncontrolled infection.

Are people more likely to have genetic deficiencies for innate or adaptive immunity? Why?
Barriers to Infection

Figure 21.23 Physical, chemical, and anatomical barriers to infection.

- Lysozyme in tears and other secretions dissolves cell walls.
- Normal flora compete with pathogens.
- Skin is a physical barrier, produces antimicrobial fatty acids, and its normal flora inhibits pathogen colonization.
- Rapid pH change inhibits microbial growth.
- Flushing of urinary tract prevents colonization.
- Mucus, cilia lining trachea suspend and move microorganisms out of the body.
- Blood proteins inhibit microbial growth.
- Mucus and phagocytes in lungs prevent colonization.
- Stomach acidity (pH 2) inhibits microbial growth.
- Normal flora compete with pathogens.
- Removal of particles including microorganisms by rapid passage of air over cilia in nasopharynx.
Microenvironments

- Bacteria colonize your body and modify their environment to prevent colonization by other microbes.
- Change in pH (skin, genital tract, etc.)
- Anaerobic bacteria in your mouth (hence plaque)
A Few Cellular Components of Innate Immunity

- **Neutrophils**
  - Phagocytic, short-lived

- **Macrophages**
  - APC, long-lived, stimulate innate and adaptive immune responses

- **NK (Natural Killer) Cells**
  - derived from same lineage as B and T cells
Neutrophils

- Variable number and shape of nucleus
- Not found in healthy tissue
- Signalled by macrophages to come to infected site and there become dominant phagocyte

- **Pathogen Associated Molecular Patterns** recognized by neutrophils:
  - Mannose
  - LPS (Lipopolysaccharide)
  - Flagellin
Macrophages

- Are covered in surface receptors that recognize PAMPs
- Important APCs that coordinate innate and adaptive immune response
- Release cytokines to stimulate other cells

*Figure 2-5 Immunobiology, 6/e. (© Garland Science 2005)*
# Bactericidal Agents Produced by Phagocytes

<table>
<thead>
<tr>
<th>Class of mechanism</th>
<th>Specific products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidification</td>
<td>pH=3.5–4.0, bacteriostatic or bactericidal</td>
</tr>
<tr>
<td>Toxic oxygen-derived products</td>
<td>Superoxide $\text{O}_2^-$, hydrogen peroxide $\text{H}_2\text{O}_2$, singlet oxygen $^1\text{O}_2^*$, hydroxyl radical $\text{OH}^-$, hypohalite $\text{OCl}^-$</td>
</tr>
<tr>
<td>Toxic nitrogen oxides</td>
<td>Nitric oxide NO</td>
</tr>
<tr>
<td>Antimicrobial peptides</td>
<td>Defensins and cationic proteins</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Lysozyme—dissolves cell walls of some Gram-positive bacteria. Acid hydrolases—further digest bacteria</td>
</tr>
<tr>
<td>Competitors</td>
<td>Lactoferrin (binds Fe) and vitamin B$_{12}$-binding protein</td>
</tr>
</tbody>
</table>

Figure 2-6 Immunobiology, 6/e. (© Garland Science 2005)
Cytokine = proteins made by cells that affect the behavior of other cells. Bind to a specific receptor on the target cell.
Sepsis

• Results from a loss of blood pressure and vascular integrity

• Death occurs from organ failure

• An overreaction of the immune system, a prime example of responses to pathogens that can kill the host

Figure 2-45  Immunobiology, 6/e. (© Garland Science 2005)
### Mammalian TLRs

- Different **Toll-like receptors** bind to various **PAMPs**.

<table>
<thead>
<tr>
<th>Toll-like receptor</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR-1 dimer</td>
<td>Peptidoglycan, Lipoproteins, Lipoarabinomannan (mycobacteria), GPI (T. cruzi), Zymosan (yeast)</td>
</tr>
<tr>
<td>TLR-2/TLR-6 dimer (plus CD14)</td>
<td>LPS (Gram-negative bacteria)</td>
</tr>
<tr>
<td>TLR-3</td>
<td>dsRNA</td>
</tr>
<tr>
<td>TLR-4 dimer</td>
<td>LPS (Gram-negative bacteria)</td>
</tr>
<tr>
<td>TLR-5</td>
<td>Flagellin</td>
</tr>
<tr>
<td>TLR-9</td>
<td>Unmethylated CpG DNA</td>
</tr>
</tbody>
</table>
Interferon

- Cytokine that induces an anti-viral state in other cells
- Stimulated by TLR-3, which binds to dsRNA
- Also degrades intracellular RNA and increase protease activity
Figure 15-3  Immunobiology, 6/e. (© Garland Science 2005)
TLR

The mammalian Toll-like receptor signaling pathway

Toll

The Drosophila Toll signaling pathway

TNFR

The Drosophila Imd pathway detects Gram-negative bacteria through a pathway analogous to mammalian TNF receptor pathway

Imd

The mammalian TNFR pathway

The Drosophila Imd pathway

Figure 15-4 Immunobiology, 6/e. (© Garland Science 2005)

Figure 15-5 Immunobiology, 6/e. (© Garland Science 2005)
**Toll** is stimulated by a host-protein that is cleaved after encountering a pathogen.

**TLRs** are stimulated by direct pathogen-receptor interaction.
• Homologous proteins exist in both pathways.

• Both can result in apoptosis
Natural Killer Cells

- Some viruses downregulate MHC-1 expression on infected cells

- **NK cells** induce apoptosis in cells missing MHC-1

---

Figure 2-50 Immunobiology, 6/e. (© Garland Science 2005)
Complement System

- Secreted as inactive enzymes known as **zymogens** (enzymes that must be modified in order to be active)
- Plasma proteins that attack extra-cellular pathogens
- Being coated in Complement can result in:
  - Phagocytosis
  - MAC (Membrane-Attack Complex)
Complement Pathways

**Classical Pathway**
- Antigen:antibody complexes (pathogen surfaces)
  - C1q, C1r, C1s
  - C4
  - C2

**MB-Lectin Pathway**
- Mannose-binding lectin binds mannose on pathogen surfaces
  - MBL, MASP-1, MASP-2
  - C4
  - C2

**Alternative Pathway**
- Pathogen surfaces
  - C3
  - B
  - D

**C3 Convertase**
- C3a, C5a
  - Peptide mediators of inflammation, phagocyte recruitment
- C3b
  - Binds to complement receptors on phagocytes

**Terminal Complement Components**
- C5b
- C6
- C7
- C8
- C9
  - Membrane-attack complex, lysis of certain pathogens and cells
  - Opsonization of pathogens
  - Removal of immune complexes

Figure 2-19 Immunobiology, 6/e. (© Garland Science 2005)
The Alternative Pathway

Complement proteins bind to pathogen surfaces, which are unable to repel the attack.

This coating with C3b signals macrophages and neutrophils to phagocytize the pathogen.

Host cells have regulatory proteins to prevent this cascade.
Spontaneous formation of **C3 Convertase**, which converts C3 into C3a and C3b

**C3b** binds to pathogen surfaces

**C3a** is cleaved and mediates inflammation

Why is inflammation good?
Small complement-cleavage products act on blood vessels to increase vascular permeability and cell-adhesion molecules.

C3a, C5a, C4a

Increased permeability allows increased fluid leakage from blood vessels and extravasation of immunoglobulin and complement molecules.

IgG, IgM

Migration of macrophages, polymorphonuclear leukocytes (PMNs), and lymphocytes is increased. Microbicidal activity of macrophages and PMNs is also increased.
Factor B binds noncovalently to C3b on a cell surface and is cleaved to Bb by factor D.

On host cells, the complement-regulatory proteins CR1, H, MCP, and DAF bind to C3b. CR1, H, and DAF displace Bb.

Pathogens lack complement-regulatory proteins. Binding of properdin (factor P) may stabilize the C3bBb complex.
Host

C3b bound to H, CR1, and MCP is cleaved by factor I to yield inactive C3b (iC3b)

Pathogen

C3bBb complex is a C3 convertase and deposits many molecules of C3b on the pathogen surface

No activation of complement on host cell surfaces

Opsonization, activation of terminal complement components

Figure 2-26 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)
Mannose on bacterial cells stimulates MB-lectin to deposit C3b on pathogen which forms a C3 Covertase.
Classical Complement Pathway

Pentameric IgM molecules bind to antigens on the bacterial surface and adopt the 'staple' form

'IgG molecules bind to antigens on the bacterial surface

C1q binds to one bound IgM molecule

C1q binds to at least two IgG molecules

Binding of C1q to Ig activates C1r, which cleaves and activates the serine protease C1s

Figure 9-28 Immunobiology, 6/e. (© Garland Science 2005)
• Once complement (C1s) binds to antibodies, it stimulates a cascade to build **C3 Convertase** which coats the pathogen in **C3b**.

• This results in **phagocytosis** and/or **MAC** formation
The components of a primitive complement system in echinoderms

factor B  C3
factor D  factor I

?  ?

C3 receptor

bacterium
Why are most of the viruses that have evolved resistance in the Poxviridae, Herpesviridae, and Coronaviridae families?

And now for something completely different...
Hypermutation as a Defense Against Retroviruses

- Hypermutation in B-cells is caused mainly by AID (Activation-Induced Cytidine Deaminase)
- Causes all sorts of mutations during Affinity Maturation, mainly C to T
- HIV genomes have been found with increases in G to A mutations
• A gene coding for a protein closely related to AID, **APOBEC3G**, has the ability to hypermutate retroviruses.

• Prevents initiation of infection.
• Why could this be a very good thing?

Natural Selection = an already existing function that is co-opted for a novel use (somatic hypermutation to antiretroviral function)
• If \textit{vif} is not present, or not effective, APOBEC3G will be incorporated into the budding virus.
• When the virus infects a new cell and undergoes reverse-transcription, APOBEC3G will deaminate the new DNA strand.
Cytidine Deamination of a Retrovirus

Normal Reverse Transcription

APOBEC3G Hypermutation
Evolution of AID Gene Family

- Genes are very ancient (found in *Xenopus* & Fugu)

- Massive expansion of gene family in primates, especially human lineage

Evolution of AID Gene Family

- Gene duplication
- Genomic movement
- 3A, 3F, and 3G all have documented antiretroviral function

Sawyer et al. (2004) PLOS Biol e275
Controlling lentiviruses: Single amino acid changes can determine specificity

<table>
<thead>
<tr>
<th>Apobec3G</th>
<th>Vif</th>
<th>HIV-1</th>
<th>SI Vagu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo Sapiens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D128</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>D128K</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Chlorocebus aethiops</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K128</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>K128D</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

- Species-specific APOBEC3G blocks infection with virus from other species
- Not even have the chance to evolve in the new host

Phylogeny of HIV and SIV

Gordon et al. (2005) LANL HIV Database Review Articles
Positive Selection on APOBEC3G

Natural selection favors rapid change in protein sequence.

Leads to rapidly divergent genes between species.

Happened in almost every OWM and Ape lineage.

Sawyer et al. (2004) PLOS Biol e275