Innate Immunity

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Lecture outline

• General features of innate immunity
• Components of the innate immune system
• Foreign and self molecules that stimulate innate immune responses
• Recognition molecules
  • TLRs, NLRs, Inflammasomes, RNA and DNA sensors
• Innate effector responses
  • Inflammation
  • Anti-viral state
• Innate immunity stimulation of adaptive immunity
General features of innate immunity

- Phylogenetically ancient (evolved before adaptive immunity)
- Functional even before exposure to microbes (no prior sensitization/immunization needed)
- First responders that eliminate some infections, or hold at bay other infections until adaptive immunity kicks in
- Limited types of induced responses:
  - Inflammation
  - Antiviral state
- Resets to baseline (limited memory)
  - Some evidence for macrophage and NK memory
- Stimulates adaptive immunity
  - Innate immunity provides “danger signals”
Components of the Innate Immune System: Cells

- **Epithelial barriers**
  - Mechanical barrier
  - Locally produced antibiotics

- **Sentinels**
  - Dendritic cells
  - Mast cells
  - Tissue resident macrophages

- **Recruited Phagocytes**
  - Macrophages
  - Neutrophils

- **Specialized lymphocytes**
  - Innate lymphoid cells (ILCs): Cytokine producers
  - NK T cells, γδ T cells, MAIT cells
Two Sources of Macrophages

Recruited monocyte-derived macrophages: Inflammation

Tissue resident macrophages: Homeostasis Sentinel role

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Components of the Innate Immune System: Soluble proteins

- **Plasma proteins**
  - Complement
  - Ficolins (lectins)
  - Collectins (Mannose Binding Lectin)
  - Pentraxins (C Reactive Protein, serum amyloid protein)

- **Cytokines**
  - Inflammatory (IL-1, TNF, IL-6, IL-12)
  - Chemokines (CXCL8, CCL2, many others)
  - Anti-viral (Type 1 interferons: IFN α, IFN β)
Complement Pathways

**Initiation of complement activation**

- **Innate**
  - Alternative pathway
  - Classical pathway
  - Lectin pathway
  - Mannose binding lectin

- **Adaptive**
  - Classical pathway
  - C1
  - C2, C4

- **Early steps**
  - C3, C3
  - C3b
  - C3b deposited on microbe
  - C5

- **Late steps**
  - Membrane attack complex (MAC)
  - C5a
  - Lysis of microbe
  - H2O

**Effector functions**

- C3a: Inflammation
- C3b: Opsonization and phagocytosis
- C5a: Inflammation

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Innate Immune System: What is recognized?

• Structures that are shared by various classes of microbes but are not present on host cells - Pathogen associated molecular patterns (PAMPs).
  – Innate immunity often targets microbial molecules that are essential for survival or infectivity of microbes (prevents escape mutants)

• Structures produced in damaged or necrotic host cells - Damage associated molecular patterns (DAMPs).
Cellular Pattern Recognition Receptors

All cellular compartments and all microbe types are covered

5 major classes

- **TLRs**: Toll like receptors
- **CLR**: C-type lectin receptors
- **NLR**: NOD-like receptors
- **RLR**: RIG like receptors
- **CDS**: Cytosolic DNA sensors
# Innate vs Adaptive Immune Recognition

<table>
<thead>
<tr>
<th>Structures recognized</th>
<th>~1,000</th>
<th>&gt;10⁷</th>
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<tbody>
<tr>
<td>Different microbes</td>
<td></td>
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<tr>
<td>Identical mannose receptors</td>
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<table>
<thead>
<tr>
<th>Receptors</th>
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<tbody>
<tr>
<td>TLRs</td>
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<tr>
<td>NLRs</td>
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<tr>
<td>CLRs</td>
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<tr>
<td>Collectins</td>
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<td>Pentraxins</td>
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~ 100 types; Limited variation of each type

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<th>Distribution of receptors on cells</th>
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<tr>
<td>Non-clonal</td>
</tr>
<tr>
<td>Clonal</td>
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Two types; Millions of variations of each type
Toll-like Receptors: Specificity Location
Toll-like Receptor signaling

NF-κB induced expression of proinflammatory genes:
• Cytokines (TNF, IL-1, IL-6, IFN-α)
• Chemokines
• Endothelial adhesion molecules

Acute inflammation

IRF7/3 induced expression of Type 1 interferon genes
• IFN-α
• IFN-β

Anti-viral state

NF-κB induced expression of costimulatory molecules
• CD80
• CD86

Stimulation of T cells / adaptive immunity
Toll-like Receptors (TLRs): Clinical Relevance

• Excessive/systemic TLR signaling underlies pathophysiology of sepsis (LPS/TLR4; Peptidoglycan/TLR2)

• TLR signaling in B cells promotes auto-antibody production

• Gain of function MyD88 mutations drive B cell lymphomas

• TLR ligands, such as CpG nucleotides, are potentially useful adjuvants to enhance effectiveness of vaccines
NOD-like receptors (NLRs)*

• A family of >20 cytosolic proteins, best known:

• NOD1 and NOD2
  – Recognize derivatives of bacterial peptidoglycan **; Activate NF-kB and trigger inflammation

• NLRPs
  – NLRs that contain “pyrin” domains
  – Sense diverse DAMPs and PAMPs
  – Form signaling complex called the inflammasome, which leads to the production of IL-1 and inflammation**

*NOD = nucleotide oligomerization domain; **DAP , Diaminopimelic acid; MDP, muramyl dipeptide; ***n.b. not all inflammasomes use NLRPs
**Inflammasomes**

**Main components:**
- Sensor
- Adaptor
- Caspase 1

**Main functions:**
- Caspase-1 mediated processing and release of Interleukin-1 (IL-1)
- Pyroptosis: Gasdermin D-mediated inflammatory cell death

**Triggers:**
- Diverse PAMPs, DAMPs, changes in cells caused by microbes
Inflammasomes/IL-1 in rare and common inflammatory diseases

- Gain of function mutations in inflammasome components cause rare inherited “auto-inflammatory” syndromes
  - Constitutive activation and uncontrolled IL-1 production
  - IL-1 antagonists are very effective treatments for these disorders.

- Gout, pseudogout: Deposition of crystals (e.g. urate) → IL-1-mediated acute inflammation

- Deposition of cholesterol crystals → role of inflammation in atherosclerosis (question of clonal hematopoiesis association with CVD)

- Others
DNA sensing: the cGAS STING pathway

There are many DNA sensors in the cytosol of cells.*

cGAS is a major sensor

STING mediates the function of most of these DNA sensors

Main signaling function of STING is IRF3-dependent type 1 interferon antiviral response

STING pathway may also be activated by damaged tumor DNA-? role in tumor immunity

* cGAS, DDX41, DAI, RNA polymerase III, IFI16
RNA sensing: Rig-like Receptors (RLRs)

The two main RLRs are RIG-I and MDA5

Recognize cytosolic viral RNA and trigger a signaling pathway that leads to the activation of IRFs that stimulate type I interferons

RLRs interact with a mitochondrial membrane protein mitochondrial antiviral-signaling (MAVS)

Features of Viral RNA detected

**RIG-1**
- short ds RNA
- 5′ triphosphate or diphosphate
- absence of 2′-O-methylation of the 5′ end of nucleotides
- blunt-ended base pairing

**MDA5**
- long dsRNA >300 bp
The major reactions and functions of innate immunity

- Induction of inflammation: killing of microbes, removal of dead cells, foreign bodies
- Induction of the anti-viral state: inhibition of viral replication
- Stimulation of the adaptive immune response

Take home messages
What is Inflammation?

- A response to infection and/or injury of vascularized tissues whereby...
- Blood-derived fluid, proteins, and leukocytes accumulate, which...
- Kill and remove offending agent (e.g. microbes), remove dead cells, and repair damage
Leukocyte migration from blood into tissues

- Rolling
- Integrin activation by chemokines
- Stable adhesion
- Migration through endothelium

- Leukocyte
- Chemokine receptor
- Chemokine
- Selectin
- Integrin (low-affinity state)
- Selectin ligand
- Blood flow
- Integrin (high-affinity state)
- Proteoglycan
- Cytokines (TNF, IL-1)
- Macrophage stimulated by microbes
- Chemokines
- Fibrin and fibronectin (extracellular matrix)
Molecular basis of leukocyte migration through endothelium

• **Selectins**: low-affinity binding of leukocytes to endothelium (slows down flowing cells)

• **Chemokines**: activation of integrins to high affinity state (and chemokinesis of leukocytes in tissues to site of infection or tissue damage)

• **Integrins**: firm adhesion/arrest of leukocytes on endothelium
Actions of Cytokines in Inflammation

**Local inflammation**
- **Endothelial cells**
  - TNF, IL-1
  - Chemokines
  - Adhesion molecule
  - Increased permeability
- **Leukocytes**
  - TNF, IL-1
  - IL-1, IL-6
  - Chemokines
  - Activation

**Systemic protective effects**
- **Brain**
  - TNF, IL-1, IL-6
  - Fever
- **Liver**
  - IL-1, IL-6
  - Acute phase proteins

**Systemic pathological effects**
- **Heart**
  - TNF
  - Low output
- **Endothelial cells/blood vessel**
  - TNF
  - Thrombus
  - Increased permeability
- **Multiple tissues**
  - TNF
  - Skeletal muscle
  - Insulin resistance

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Excess Innate Cytokine Syndromes

• Pathologically abundant innate cytokine production (TNF, IL-1, IL-6) leading to systemic inflammation with organ damage, coagulopathy, and shock occurs, in various clinical syndromes, e.g.:
  • Macrophage activation syndrome: JRA
  • Cytokine release syndrome: Adoptive T cell therapy for cancers
  • Cytokine storm: SARS CoV2
  • Hemophagocytosis and lymphohistiocytosis (HLH): Perforin deficiency
  • Septic shock: infections
  • Toxic shock syndrome (TSS): bacterial infections

• In some cases excess T cell activation with IFN-γ leads to excess macrophage activation which leads to excess IL-1, TNF, IL-6

• Cytokine antagonists (e.g. mAbs specific for IL-1, IL-6R, TNF, IFN-γ) may be effective in some cases.
Induction of the Anti-Viral State:

Type 1 interferon production

- Many pathways induce IRFs
- IRFs promote Typ1 IFN transcription

The key to RNA vaccines
Induction of the anti-viral state: Functions of Type I IFNs

- Activate transcription of several genes that confer on cells a resistance to viral infection
- Sequestration of lymphocytes in lymph nodes, maximizing opportunity for encounter with microbial antigens
- Increase cytotoxicity of NK cells and CD8+ CTLs
- Promote the differentiation of naive T cells to the Th1 subset of helper T cells.
- Upregulate class I MHC expression increasing CTL recognition of virally infected cells
Importance of Type I IFN Responses: Lessons from COVID19 Patients

Inborn errors of type I IFN immunity in patients with life-threatening COVID-19

Auto-antibodies against type I IFNs in patients with life-threatening COVID-19

These two studies indicated that impaired Type 1 interferon responses increase risk for severe SARS CoV-2 infection.
Type I interferonopathies: Genetic diseases with dysregulated type 1 IFN responses
The innate immune system provides second signals required for lymphocyte activation

**Second signals for T cells:** “costimulators” induced on APCs by microbial products, during early innate response

**Second signals for B cells:** products of complement activation recognized by B cell complement receptors

*Take home messages*