Lecture on Innate Immunity and Inflammation

- Evolutionary View
- Epithelial barriers to infection
- Four main types of innate recognition molecules: TLRs, CLR, NLRs, RLRs
- NF-κB, the master transcriptional regulator of inflammation
- Inflammation and recruitment of phagocytes
- Killing of bacteria by phagocytes
- Anti-viral innate immunity: the interferon system and apoptosis of virus-infected cells

Innate Immunity: An Evolutionary View

- All multicellular organisms have defense mechanisms against microbial and viral infections
- For vertebrates, immune defense can be divided into innate immunity and adaptive immunity
- Vertebrate innate immune elements are closely related to components of immunity in invertebrates (especially TLRs and complement)
Innate Immunity: An Evolutionary View

- All multicellular organisms have defense mechanisms against microbial and viral infections
- For vertebrates, immune defense can be divided into innate immunity and adaptive immunity
- Vertebrate innate immune elements are closely related to components of immunity in invertebrates
- Innate immunity retains importance as
  - A first line of defense, slowing growth of infectious agents until adaptive immunity kicks in
  - A means of directing adaptive immunity (induction of inflammation, activation of dendritic cells, and production of cytokines that specialize immune responses)

Recognition mechanisms of innate immunity (concepts)

- What is seen by innate immunity?
  - Microbes evolve rapidly, so innate immunity must focus on highly conserved and essential components of microbes (cell wall structures; nucleic acids) “Pathogen-associated molecular patterns” (PAMPs)
  - disadvantages of the term PAMP: generally microbe-associated, not pathogen per se; “patterns” true for some more than others
**Innate immune recognition of bacterial cell wall components**

**Gram-negative bacteria**
- lipopolysaccharide
- peptidoglycan
- outer membrane
- lipoprotein
- plasma membrane

**Gram-positive bacteria**
- teichoic acid
- peptidoglycan
- lipoteichoic acid
- plasma membrane

**Recognition mechanisms of innate immunity (concepts)**

- What mediates the recognition of PAMPs?
  - Diverse recognition elements; 4 key families of “Pattern Recognition Receptors” (2 for extracellular, vesicles; 2 for cytoplasm)
    - Toll-like receptors (TLRs; transmembrane receptors)
    - C-type lectin receptors (CLRs; transmembrane receptors)
    - NOD-like receptors (NLRs; cytoplasmic sensors)
    - RigI-like receptors (RLRs; cytoplasmic RNA helicases)
  - Also, recognition of molecules released from necrotic cells, tissue damage (“damage associated molecular patterns” DAMPs or “danger”) TLRs, CLRs, NLRs
  - New hypothesis: recognition of perturbations induced by pathogens (“patterns of pathogenicity”) such as bacterial pore-forming toxins, perturbations of the cytoskeleton, various types of cell stress etc.)—recognition mechanisms less well understood (inflammasome, etc.)

(Lectin: a protein that binds to carbohydrates)
The Epithelial Layer: The initial barrier to infection

1. Physical barrier of the epithelial layer (toughness of barrier varies by location due to other functions: air exchange, nutrient uptake, etc.)
2. Acid pH of the stomach
3. Anti-microbial peptides secreted by some epithelial cells (small intestines, small airways of lungs)
4. Mucus/cilia to remove particles, microbes from airways: mucus layer in gut creates spatial separation between epithelial cells and most of bacteria
5. Microbe-binding molecules outside the epithelial layer: IgA; surfactants A/D (lung)

Recognition of an infection once it gets past the epithelial barrier

- Soluble innate immune recognition elements (collectins, ficolins, complement)
- Sentinel innate immune cells of tissues: tissue macrophages, mast cells and immature dendritic cells
Soluble innate recognition and complement activation

Mannose binding lectin
Lung surfactants A, D
Ficolins

“pattern recognition receptors”; in this case pattern of terminal sugars on cell surfaces

Activation of the Complement Pathway:
2 innate modes of initiation

Lectin Pathway
MBL, Ficolins

Classical Pathway
IgG, IgM, pentraxins

Alternative Pathway
Microbial Membrane

early events

Cleavage of C3 by C3 convertase

late events

Inflammation
Chemoattraction
(C3a, C5a)

Induction of
phagocytosis
(C3b)

Attack on
membranes
(C5b, C6, C7, C8, C9)

Promotion of
antibody production
(C3b)
Toll is required for innate defense in flies

J. Hoffmann et al. Cell 1996

Toll-like receptors and recognition of pathogens

Toll-like receptors directly bind ligands (mostly)

**TLR3**

dsRNA

*Moresco, LaVine & Beutler, Current Biology 21: R488, 2011*

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**Cellular location of TLRs**

TLRs that recognize nucleic acids are localized inside cells - thought to promote discrimination between viral and self nucleic acids

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Toll-like receptor signaling pathways

- Ligand induced dimerization of TLR--
- induced assembly with TIR-domain containing adaptors
- MyD88 pathway and TRIF pathway;
- Activate Transcription factors and MAP kinases

Pathways of NF-κB activation

NF-κB is a family of transcription factors: p50, p52, p65 (Rel-A), c-Rel, Rel-B; plus inhibitors (I-κB)

Canonical pathway

Non-canonical Pathway (activated by some TNF receptor family members)
Genes regulated by NF-κB

Inflammatory cytokines
- TNF
- IL-1
- IL-6
- IL-12
- Lymphotoxin α/β
- GM-CSF
- IFN-β

Chemokines
- IL-8
- MIP-1α
- MCP
- RANTES
- Eotaxin

Adhesion molecules
- ICAM-1
- VCAM-1
- E-selectin

Immune effector molecules
- FasL
- iNOS
- COX-2
- β-defensins

Pro-survival molecules
- Bcl-XL
- A1
- c-IAP1, 2

Innate recognition by CLRs (examples)

Myeloid DC

NOD1 & NOD2 recognize peptidoglycan substructures and promote innate immune responses

NOD1 and NOD2 are intracellular molecules and resemble some plant disease resistance proteins; best understood of the "NOD-like receptors" or NLRs.

Common alleles of NOD2 are a genetic risk factor for Crohn's disease

- Several moderately common alleles of the NOD2 gene (7% of total alleles) increase susceptibility to Crohn's disease (a form of inflammatory bowel disease)
- Two copies of these alleles increase susceptibility by 40X
- Pretty strong evidence that these alleles are "loss of function" alleles
- NOD1/2 have been shown to have 4 immune functions: activation of inflammatory cytokine gene expression; induction of anti-microbial peptide synthesis by Paneth cells in intestines; activation of inflammasome; autophagy of bacteria in cytoplasm
Processing of IL-1 and related cytokines: an important regulatory step

- Some "NLRs" assemble to form the "inflammasome" which proteolytically processes IL-1 and related cytokines to their active, secreted forms.
- Inflammasome is activated by cellular stress or recognition of microbial components in the cytoplasm.
- Genetic periodic fever syndromes are due to activating mutations in inflammasome.
- Activated by small crystals, important role in Gout.
- Suggestive evidence that inflammasome may be activated by cholesterol crystals (atherosclerotic lesions?); possible role in type 2 diabetes? Possible role in Alzheimer's disease?

The NALP3-inflammasome activates caspase 1 in response to cellular insults

- Endocytosed crystals
- Bacterial pore-forming toxins
- Efflux of K+
- Other insults/stresses

- TLRs or NOD1/NOD2 induce synthesis of pro-IL-1
- Inflammasome processes it to generate active IL-1
Inflammation

- Pro-inflammatory cytokines (TNF, IL-1) signal to endothelial cells to make them:
  - Leaky to fluid (influx of plasma; containing antibodies, complement components, etc.)
  - Sticky for leukocytes, leading to influx of neutrophils first, then monocytes, lymphocytes
  - Systemic effects: fever, acute phase response
- Inflammation may also be triggered by complement activation or by activation of the coagulation system

Inflammation: Neutrophils vs. Monocytes

- Acute inflammation is initially characterized as rich in neutrophils; later it is more monocytes. This is controlled by which chemokines are expressed by the endothelial cells. (Note: Th17-driven inflammation is neutrophil-rich).
- Neutrophils are dedicated to killing bacteria and are short-lived. They often damage host tissue as a byproduct.
Inflammation: Neutrophils vs. Monocytes

- Monocytes are multi-potential, depending on cytokine signals:
  - IFN-γ: assume a vigorous killing phenotype similar to neutrophils
  - IL-4: “alternatively activated macrophages”; tissue repair, barrier immunity
  - IL-10: assume a wound-healing type phenotype (to clean up after infection is cleared)

Sepsis Syndrome

- Bacterial septicemia leads to activation of TLRs on monocytes in the blood
- Systemic release of TNF and IL-1 leads to “inflammation” all over the body
- Shock from loss of blood pressure (vasodilation and leakage of fluid into tissues)
- TLRs also induce coagulation (via tissue factor)
- The combination of effects can lead to multi-organ failure and death
Type 2 Inflammation

- Inflammation may be characterized by influx of eosinophils and basophils instead of neutrophils and monocytes
- This type of inflammation is seen in some infections with parasites (worm infections), and in asthma and allergies, as will be discussed later in the course
- This type of inflammation can be induced by innate immunity (role of iH2/IL2 cells?), or by IgE and mast cells, or by Th2 cells

Phagocytosis

binding of soluble opsonins to particle

particle binding via phagocytic receptor

particle engulfment with actin polymerization

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Opsonins and Phagocytic receptors

Opsonins
- Complement components (C3b)
- Collectins (mannose-binding lectin)
- Antibodies

Phagocytic receptors
- Receptors for opsonins (complement receptors, Fc receptors)
- Pattern recognition receptors (mannose receptor, etc.)
- Receptors for apoptotic cells

Phagocytosis
Phagocytosis and killing

Primary granules:
- Antimicrobial peptides
- Lysozyme (degrades peptidoglycan)
- Proteases (elastase, etc.)

Secondary granules:
- Phagocyte oxidase

Lysosomes:
- Digestive enzymes

Phagocyte oxidase (NADPH oxidase): makes reactive oxygen intermediates (superoxide anion, hydrogen peroxide)
Myeloperoxidase: hypochlorous acid
Inducible Nitric oxide synthase (iNOS): makes reactive nitrogen intermediates (NO)

Chronic granulomatous disease: genetic defect in phagocyte oxidase (most commonly gp91, which is X-linked)
Mice lacking both Phox AND iNOS are extremely susceptible to bacterial infection
Viral Immunity

•Viruses evolve extremely rapidly, great challenge for innate immunity
•Anti-viral immunity has 2 roles
  – Blocking infection (antibodies, complement, etc.)
  – Blocking viral replication (interferon, killing infected cells)
•Viruses have evolved many mechanisms of evading immunity

Anti-retroviral defense by a cytidine deaminase APOBEC3G

HIV Vif binds to APOBEC3G and blocks it (details of virus assembly not accurate)
Virus-infected cell produces interferon to act on neighboring cells

Virus-infected cell

Interferon-α

Infected cell makes interferon, uninfected cells respond to interferon and become refractory to viral growth

Production of interferon by infected cells

RIG-I, MDA-5: have RNA helicase domain and CARD domain ("RLRs")

Required adaptor "MAVS"
Response of cells to interferons

Anti-viral effects of interferon α/β
Plasmacytoid dendritic cells

- Many cell types produce small amounts of type 1 interferons upon infection
- There is a dendritic cell subtype ("plasmacytoid dendritic cell"; "natural interferon-producing cell") that produces 100-1000x more interferon upon contact with viruses, does not need a productive infection.
- Also produces a large amount of TNF
- Recognition mechanism: probably TLR7, TLR9
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- Recognition mechanism: probably TLR7, TLR9.
- Function? anti-viral innate immunity; stimulation of adaptive immunity?

NK cells are regulated by the balance between activating and inhibitory receptors.

NK cell has activating receptors and inhibitory receptors: killing believed to occur if activating receptors dominate (relative numbers of ligands on target cell).

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Recognition mechanisms of innate immunity
(summary of examples)

Toll-like receptors (TLRs):
- bacterial cell wall components, viral nucleic acids

Collectins, mannose receptor (CLR)s:
- distinctive cell surface polysaccharides

Alternative pathway of complement:
- cell surfaces lacking protective complement inhibitory proteins

Anti-microbial peptides:
- acidic phospholipids on outside of membrane

Intracellular NLRs:
- peptidoglycan; cellular stress

Interferon-induction (RLRs):
- double-stranded RNA (replication of viral genome)

Virus replication-induced cell stress:
- induction of apoptosis, expression of stress-induced molecules that alert NK cells