Innate Immunity, Inflammation and Toll-like Receptors (TLRs)

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Overview

I. Inflammation and the Immune Response

II. Positive and Negative Outcomes of and Immune Response

III. Toll-like Receptor (TLR) Biology

IV. Innate Immunity in the CNS
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Immune System

A system of defenses by which the body (host) recognizes self from non-self (foreign material)

The immune system destroys or neutralizes foreign matter, both living and nonliving.
White Blood Cells are Mediators of the Immune Response

White Blood Cell Lineages

Bone Marrow

heteroplastic stem cell
The Immune Response to Infectious Pathogens

Infectious Pathogens

Parasitic worms  Extracellular bacteria, parasites, fungi  Intracellular bacteria, parasites  Viruses
(extracellular)  (extracellular)  (intracellular)

cutaneous/mucosal membrane

Innate Immune Response

Adaptive Immune Response
Both Innate and Adaptive Immunity Depend on the Activities of White Blood Cells

Innate Immune Response
- neutrophils
- macrophages

Adaptive Immune Response
- dendritic cells "DC"
- lymphocytes
Innate Immune Response
- Immediate response
  0-96 hours
- Targets
groups of pathogens
- No Memory

Adaptive Immune Response
- Gradual response
  > 96 hours
- Targets
  specific pathogens
- Memory

Pathogenic Microbes
- Parasitic worms (extracellular)
- Extracellular bacteria, parasites, fungi
- Intracellular bacteria, parasites
- Viruses (intracellular)
- Parasitic worms (extracellular)
- Macrophage
- Neutrophil
- Lymph node
- B cell
- T cell
- Monocyte
- Immature dendritic cells
Innate Immunity

The First Line of Defense

Innate Immune Cells
Recognition of Pathogens by Innate Immune Cells

initiated when innate immune cell

Pattern Recognition Receptors

including Toll-like receptors (TLRs), Nod-like receptors (NLRs) and RIG-like receptors (RLRs)

are triggered by microbe-specific motifs,

Pathogen-Associated Molecular Patterns (PAMPs)

Events Elicited by Triggering of Macrophage and Neutrophil TLRs

- phagocytosis
- secretion of inflammatory cytokines
- secretion of chemokines (chemoattractants); recruitment of additional innate immune cells
Ilya Illich (a.k.a. Elie) Mechnikov
First Observed Phagocytosis by Phagocytes
a fundamental process of the innate immune response

Phagocytosis
Microbe or other foreign material taken up by endocytosis and isolated and destroyed within a phagolysosome

Vander's Human Physiology. The McGraw-Hill Companies, Inc., Editors
### Agents produced or released by *phagocytes* on ingestion of microorganisms

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acidification</strong></td>
<td>pH 3.5–4.0 bacteriostatic, bacteriocidal</td>
</tr>
<tr>
<td><strong>Toxic O₂-derived products</strong></td>
<td>Superoxide (O₂⁻), H₂O₂, hydroxyl radical (OH⁺)</td>
</tr>
<tr>
<td><strong>Toxic nitrogen oxides</strong></td>
<td>Nitric oxide (NO)</td>
</tr>
<tr>
<td><strong>Antimicrobial peptides</strong></td>
<td>Defensins, cationic proteins</td>
</tr>
<tr>
<td><strong>Enzymes</strong></td>
<td>Lysozyme, acid hydrolases</td>
</tr>
<tr>
<td><strong>Competitors</strong></td>
<td>Lactoferrin (binds Fe), vitamin B₁₂-binding protein</td>
</tr>
</tbody>
</table>

### Secretion of Inflammatory Cytokines and Chemokines
Cytokines

- secreted in response to an activating stimulus
  - stimulate cellular effector functions
    (e.g., bacteriocidal activity of macrophages)
- induce responses by binding to specific receptors
  - autocrine acting
  - paracrine acting
  - endocrine acting

Chemokines

- class of cytokines
- chemoattractant properties
- induce cells with appropriate chemokine receptors to migrate toward the chemokine source
Acute Inflammatory Events During Innate Immune Response to Infection

1. **Vasodilation** of the microcirculation leading to increased blood flow to the infected area

2. **Increased permeability** of capillaries and venules with diffusion of blood proteins and filtration of fluid into the interstitial spaces

   Above events occur within seconds to minutes of infection. Subsequently........

3. **Chemotaxis** with movement of leukocytes from venules into the interstitium of the infected area

4. **Destruction** of pathogens in the tissues by phagocytosis and other mechanisms

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**Acute Inflammation**

*classic signs and symptoms*

- Redness
- Heat
- Swelling
- Pain
Bacteria trigger macrophages to release cytokines & chemokines

Vasodilation and increased vascular permeability cause redness, heat & swelling

Inflammatory cells migrate into tissue, releasing inflammatory mediators causing pain

Adapted from: Immunobiology. Janeway, Charles A.; Travers, Paul; Walport, Mark; Shlomchik, Mark. New York and London: Garland Science; c2001

Important Cytokines Secreted by Pathogen Activated Macrophages

- interleukin-1 (IL-1)
- interleukin-6 (IL-6)
- TNF-α
- interleukin-12 (IL-12)
- interleukin-8 (IL-8)
**Cytokine Secretion by Activated Macrophages**

Endothelial activation
Vasodilation
Lymphocyte activation
Local tissue destruction

Endothelial activation
Vasodilation
Lymphocyte activation
Increased antibody production

Chemotactic factor
Recruits neutrophils
T cells

Promotes inflammatory Th1 cell generation
Activates NK cells

Adapted from: Immunobiology, Janeway, Charles A.; Travers, Paul; Walport, Mark; Shlomchik, Mark
New York and London: Garland Science; c2001

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**Inflammation**

Chemical agent
Physical agent
Pathogenic microorganism
Tissue injury

Mediators of inflammation

Capillary dilatation
Increased capillary permeability
Attraction of leukocytes
Systemic response

Increased blood flow
Extravasation of fluid
Migration of white cells to site of injury
Fever

Heat
Redness
Tenderness
Swelling
Pain

Vander's Human Physiology, The McGraw-Hill Companies, Inc., Editors
Note the inflammation of the oropharynx and small red areas of hemorrhage (petechiae). Strep throat is caused by group A *Streptococcus* bacteria which can spread through direct contact with persons who are infected.

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Inflammation Associated with Strep Throat

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Summary of the Innate Response to an Invading Pathogenic Microbe

**macrophage**
- TLRs and other pattern recognition receptors bind pathogenic microbe motifs trigger macrophage to *phagocytize* and *destroy* infecting microbe
- activated macrophages secrete *chemokines* that attract additional innate immune cells neutrophils & monocytes

**neutrophil**
- primary cell seen early in response to pathogens
- phagocytize and destroy invading microbes

**monocytes**
- rapidly differentiate into macrophages adding to the defenses
Adaptive Immunity

The Backup Line of Defense

Adaptive Immune Cells
Antigen

that which is recognized by
the adaptive immune system
Overview of Lymphocyte Activation, Proliferation, Differentiation

Antigen stimulation

Resting naïve T cell & B cell

→ Proliferation

→ Differentiation

Memory T cell & B cell (circulating)

Effector T cell & B cell

CD4⁺ T helper cell (Th cell)

CD8⁺ cytotoxic T cell (CTL)

Plasma B cell antibody-producing

Viral Antigen Presentation

Immature mDC

→ mDC maturation

Afferent lymphatics

Effector CTL

Memory CTL

Naïve Th cell

Mature mDC

T cell differentiation

Effector Th cell

Memory Th cell

Antibody production

Plasma cell

B cell differentiation

DC: dendritic cell  Th cell: T helper cell  CTL: cytotoxic T cell (lymphocyte)
Adaptive Immunity has Memory

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Positive Outcomes of an Immune Response

Protection from Infectious Disease (Positive Outcome)

• natural immunity protects from reinfection
• vaccination protects from primary infection

Negative Outcomes of an Immune Response

Shock and Tissue Damage Negative Outcomes

• acute effects due to a “cytokine storm” / “cytokine surge” (endotoxic shock, SARS, Hanta, Dengue)
• chronic effects of cell mediated granuloma formation (Schistosomiasis)
• autoimmunity (Multiple Sclerosis, Systemic Lupus Erythematosus)
**The Cytokine Storm Endotoxic Shock**

Endothelial activation → Vasodilation → Increased vascular permeability → Lymphocyte activation → Increased antibody production → Chemotactic factor recruits neutrophils, T cells → Promotes inflammatory Th1 cell generation → Activates NK cells

**Chronic Schistosomiasis**

Continuing infection causing granulomatous reactions to schistosoma eggs and fibrosis in the affected organs

Egg → Granuloma formation → Fibrosis → Disease

Host Response → Collagen Deposition → Tissue Scarring → Circulatory Obstruction → Organ malfunction

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As early as 1989, Charles Janeway theorized that the innate immune system used specialized receptors to recognize infecting pathogens.


Toll Mutation Severely Reduces Survival of Adult Flies after Fungal Infection

Table 1. Survival of Dorsoventral Mutant Adults to Bacterial and Fungal Infections

<table>
<thead>
<tr>
<th>Genotype Tested</th>
<th>Fungal Infection</th>
<th>Bacterial Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr^R</td>
<td>69 (4,2;9)</td>
<td>95 (5,3;14)</td>
</tr>
<tr>
<td>dll/dll^f</td>
<td>74 (4,3;5)</td>
<td>92 (5,0;6)</td>
</tr>
<tr>
<td>pr^f per/ppl^f</td>
<td>4 (7,4;5)</td>
<td>87 (8,5;8)</td>
</tr>
<tr>
<td>tub^+/tub^f</td>
<td>3 (5,3;6)</td>
<td>71 (2,7;4)</td>
</tr>
<tr>
<td>Ty^f[Tj]</td>
<td>80 (10,9;8)</td>
<td>93 (6,6;9)</td>
</tr>
<tr>
<td>spz[Tfz]-spz^f</td>
<td>3 (5,6;7)</td>
<td>84 (11,5)</td>
</tr>
<tr>
<td>ee^f lee^f</td>
<td>98 (8,8;5)</td>
<td>87 (5,7;8)</td>
</tr>
<tr>
<td>imd/md</td>
<td>93 (5,6;5)</td>
<td>8 (7,4;13)</td>
</tr>
<tr>
<td>imd/md, Ty[Tj]</td>
<td>1 (2,3;5)</td>
<td>3 (4,4;6)</td>
</tr>
</tbody>
</table>

Germinating fungal hypha on a drosophila deficient for a Toll receptor gene
Toll Receptors

- best-defined pattern recognition receptors of innate immune system
  (others include Nod-like receptors [NLRs] and RIG-like receptors [RLRs])

- **Toll receptor** stimulation triggers production of anti-fungal peptides in response to fungal infections

- different Toll family members are involved in activating an anti-bacterial and anti-viral responses

Toll-like Receptors

TLRs

Mammalian homologues of drosophila Toll receptors
**Toll-like Receptors (TLRs)**

*bacterial lipopolysaccharide, LPS*

- cell-wall component of gram-negative bacteria
- can induce a dramatic systemic reaction known as *endotoxic shock*

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**Mutant Mice with TLR4 Gene Mutation**

- *unresponsive to bacterial lipopolysaccharide, LPS*
  - cell-wall component of gram-negative bacteria
- *protected from endotoxic shock*

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Defective LPS Signaling in C3H/HeJ and C57BL/10ScCr Mice: Mutations in Tlr4 Gene

<table>
<thead>
<tr>
<th>TLR</th>
<th>Exogenous Ligand; Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>tri-acetylated lipopeptides, porins; <em>Gram positive and negative bacteria</em></td>
</tr>
<tr>
<td>TLR2</td>
<td>lipopeptides, peptidoglycans, glycolipids, polysaccharides; <em>virus, Gram positive bacteria, yeast</em></td>
</tr>
<tr>
<td>TLR3</td>
<td>double-stranded RNA (dsRNA); <em>viruses</em></td>
</tr>
<tr>
<td>TLR4</td>
<td>LPS (lipid A); <em>Gram-negative bacteria</em></td>
</tr>
<tr>
<td>TLR5</td>
<td>flagellin; <em>bacteria</em></td>
</tr>
<tr>
<td>TLR6</td>
<td>di-acetylated lipopeptides; <em>Gram positive bacteria</em></td>
</tr>
<tr>
<td>TLR7</td>
<td>single-stranded RNA (ssRNA); <em>viruses</em></td>
</tr>
<tr>
<td>TLR8</td>
<td>single-stranded RNA (ssRNA); <em>viruses</em></td>
</tr>
<tr>
<td>TLR9</td>
<td>unmethylated CpG DNA; <em>bacteria, viruses</em></td>
</tr>
<tr>
<td>TLR10</td>
<td>?</td>
</tr>
</tbody>
</table>

**Human Toll-like Receptors**

**Toll-like Receptors**

- **TLRs**: plasma membrane; TLRs 1, 2, 4, 5, 6, 10
- **Endosome membrane**: TLRs 3, 7, 8, 9

**Human Toll-like Receptors**

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</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>Hsp60; Hsp70; Gp96; HMGB1</td>
</tr>
<tr>
<td>TLR2</td>
<td>Hsp60; Hsp70; Gp96; HMGB1; Fibrinogen, Surfactant protein A, Fibronectin extra domain A, Heparansulfate, defensin 2</td>
</tr>
<tr>
<td>TLR3</td>
<td>double-stranded RNA (dsRNA)</td>
</tr>
<tr>
<td>TLR4</td>
<td>Hsp60; Hsp70; Gp96; HMGB1</td>
</tr>
<tr>
<td>TLR5</td>
<td></td>
</tr>
<tr>
<td>TLR6</td>
<td></td>
</tr>
<tr>
<td>TLR7</td>
<td>single-stranded RNA (ssRNA)</td>
</tr>
<tr>
<td>TLR8</td>
<td>single-stranded RNA (ssRNA)</td>
</tr>
<tr>
<td>TLR9</td>
<td>DNA, DNA-containing immuncomplexes</td>
</tr>
<tr>
<td>TLR10</td>
<td></td>
</tr>
</tbody>
</table>

**Diagram:**

- Stress induced proteins
- Pathogens
- Chemokines
- Anti-microbial proteins
- Epigenetic modifications
- Pro-inflammatory cytokines
- Coregulatory molecules

**Inhibitory feedback**

**Persistent activation**

**Chronic Inflammation**

Systemic Lupus Erythematosus (SLE, Lupus)

- progressively debilitating, systemic autoimmune disease
- affects >5 million people worldwide
- disproportionally affects women of childbearing age
- affected males often experience severe disease

Both B cells and T cells Mediate Tissue Damaging Inflammation in SLE

- auto-antibody (Ab) production by B cells & immune complex deposition result in tissue inflammation and destruction
  - auto-reactive T cells also cause inflammatory tissue damage
- kidney damage (glomerulonephritis) leads to kidney (renal) failure
Type I Interferon Paradigm in SLE

TLR-Induced Type I IFNs promote the autoimmune disease cycle

Environmental Insult (e.g. RNA or DNA virus) → IFN-α

TLR7 & TLR9 Plasmacytoid Dendritic Cell (pDC)

Self Antigen (Ag) (cell debris) → Auto-antibody (Ab) → Auto-reactive-inflammatory Helper T cells (Th cells)

Autoimmune Disease with Organ (e.g. Kidney) Injury → Immune Complexes

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Sites of Immune Privilege

- Eye
- Testis
- CNS

Microglia

- resident innate immune cells of the CNS
- myeloid derived immune sentinels
  - express variable levels of TLR2, TLR3, and TLR4
Microglia

• recognize both pathogen and host-derived ligands in the CNS

TLR-induced activation of microglia
• positive outcomes
CNS homeostasis and immunity

Microglial PRRs Recognize Neurotoxic & Pro-inflammatory Ligands
Microglial Activation Results in Generation of Reactive Oxygen Species (ROS)

Microglia
• recognize both pathogen and host-derived ligands in the CNS

TLR-induced activation of microglia
• positive outcomes
CNS homeostasis and immunity
• negative outcomes: neurotoxicity contributing to various CNS diseases (chronic demyelinating diseases)