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

Jean Maguire van Seventer, VMD
Department of Environmental Health
Boston University School of Public Health

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
The Immune Response to Infectious Pathogens

Infectious Pathogens

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

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

Bone Marrow
- common lymphoid progenitor
- common myeloid progenitor

Thymus
- B cell
- T cell

Blood
- neutrophil polymorphonuclear
- monocyte
- immature dendritic cell

Tissue
- macrophage
- immature dendritic cell

Lymph Node
- mature dendritic cell
**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

**The Immune Response**
- Pathogenic Microbes
  - Parasitic worms (extracellular)
  - Extracellular bacteria, parasites, fungi
  - Intracellular bacteria, parasites
  - Viruses (intracellular)
- cutaneous/mucosal membrane
- immature dendritic cells
- macrophage
- neutrophils
- monocytes
- lymph node
- B cell
- T cell
- lymph node

**Innate Immune Response**

**Adaptive Immune Response**

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 Ilich (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
### Agents produced or released by phagocytes on ingestion of microorganisms

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidification</td>
<td>pH 3.5-4.0 bacteriostatic bacteriocidal</td>
</tr>
<tr>
<td>Toxic O$_2$-derived products</td>
<td>superoxide O$_2^-$  H$_2$O$_2$  hydroxyl radical OH$^+$</td>
</tr>
<tr>
<td>Toxic nitrogen oxides</td>
<td>nitric oxide NO</td>
</tr>
<tr>
<td>Antimicrobial peptides</td>
<td>defensins  cationic proteins</td>
</tr>
<tr>
<td>Enzymes</td>
<td>lysozyme  acid hydrolases</td>
</tr>
<tr>
<td>Competitors</td>
<td>lactoferrin (binds Fe) vitamin B$_{12}$-binding protein</td>
</tr>
</tbody>
</table>

### Secretion of Inflammatory Cytokines and Chemokines
**Cytokines**

- secreted in response to an activating stimulus
  - stimulate cellular effector functions
    (eg. 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**
Inflammation

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

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
Increased vascular permeability

Lymphocyte activation
Increased antibody production

Chemotactic factor
Recruits neutrophils and 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
Pathogenic microorganism
Physical agent
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 leukocytosis

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.

### 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
Adapted Immunity

The Backup Line of Defense

Adaptive Immune Cells

Bone Marrow

common lymphoid progenitor

Thymus

B cell

T cell

Bone Marrow

common myeloid progenitor

Blood

neutrophil

monocyte

monocytic cell

Immature dendritic cell

Mature dendritic cell

Lymph Node

macrophage

Immature dendritic cell

Mature dendritic cell
Antigen

that which is recognized by
the adaptive immune system

Antigen Binding Site of
Antibody and T-cell Receptor  Molecules
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

DC: dendritic cell   Th cell: T helper cell   CTL: cytotoxic T cell (lymphocyte)
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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
  - TNF-α
  - IL-8

- Vasodilation
  - IL-6

- Lymphocyte activation
  - Increased vascular permeability

- Local tissue destruction

- Increased antibody production

- Chemotactic factor
  - Recruits neutrophils and 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**

The Dorsoventral Regulatory Gene Cassette *spätzle/Toll/cactus* Controls the Potent Antifungal Response in Drosophila Adults

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>Cyo^6^</td>
<td>89 (4.2; 9)</td>
<td>96 (5.3; 14)</td>
</tr>
<tr>
<td>dll/dll^-</td>
<td>94 (4.3; 6)</td>
<td>92 (3.0; 6)</td>
</tr>
<tr>
<td>ps^2^-toll^-</td>
<td>4 (7.4; 5)</td>
<td>87 (8.5; 8)</td>
</tr>
<tr>
<td>tub^4^tub^4^-</td>
<td>3 (5.3; 6)</td>
<td>71 (27.4)</td>
</tr>
<tr>
<td>Tp^4^-Tp^4^-</td>
<td>8 (10.6; 8)</td>
<td>93 (6.6; 9)</td>
</tr>
<tr>
<td>sas^2^-sas^2^-</td>
<td>3 (5.6; 7)</td>
<td>84 (11; 9)</td>
</tr>
<tr>
<td>eastr^2^-esat^2^-</td>
<td>98 (8.8; 5)</td>
<td>87 (5.7; 8)</td>
</tr>
<tr>
<td>imd/imd</td>
<td>93 (5.6; 6)</td>
<td>8 (7.4; 13)</td>
</tr>
<tr>
<td>imd/imd, Tp^4^-Tp^4^-</td>
<td>1 (2.3; 5)</td>
<td>3 (4.4; 6)</td>
</tr>
</tbody>
</table>

**Germinating fungal hyphe 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*

Defective LPS Signaling in C3H/HeJ and C57BL/10ScCr Mice: Mutations in *Tlr4* Gene
Human Toll-like Receptors

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

Toll-like Receptors

TLRs

Diagram showing the locations of TLRs on the cell membrane and in the endosome:
- Plasma membrane: TLRs 1, 2, 4, 5, 6, 10
- Endosome membrane: TLRs 3, 7, 8, 9

Diagram showing the interactions of ligands with TLRs:
- diacyl lipopeptides
- triacyl lipopeptides
- flagellin
- LPS
- dsRNA
- ssRNA
- CpG DNA


Presentation 2 - van Seventer

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**Human Toll-like Receptors**

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<th>TLR</th>
<th>Endogenous Ligand</th>
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</thead>
<tbody>
<tr>
<td>TLR1</td>
<td></td>
</tr>
<tr>
<td>TLR2</td>
<td>Hsp60; Hsp70; Gp96; HMGB1</td>
</tr>
<tr>
<td>TLR3</td>
<td>double-stranded RNA (dsRNA)</td>
</tr>
<tr>
<td>TLR4</td>
<td>Hsp60; Hsp70; Gp96; HMGB1; Fibrinogen, Surfactant protein A, Fibronectin extra domain A, Heparansulfate, defensin 2</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 immunocomplexes</td>
</tr>
<tr>
<td>TLR10</td>
<td></td>
</tr>
</tbody>
</table>

**Diagram:**

- **CHRONIC INFLAMMATION**
- **Inhibitory feedback**
- **Persistent activation**

**References:**

Systemic Lupus Erythematosus (SLE, Lupus)

- progressively debilitating, systemic autoimmune disease
- affects >5 million people worldwide
- disproportionately 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
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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)