INNATE IMMUNITY

RAKESH SHARDA
Department of Veterinary Microbiology
NDVSU College of Veterinary Science & A.H.,
Mhow
Innate Immunity - characteristics

• Most primitive type of immune system found in virtually all multicellular animals

• high discrimination of host and pathogen

• First line of defense against infection

• no need for prolonged induction

• act quickly
  immediate direct response  0-4 hrs
  rapid induced  4-96 hrs

• antigen-independent
Innate Immunity – characteristics (contd.)

• dependence on germ line encoded receptors

• always present and active, constitutively expressed
  (some components can be up-regulated)

• Nonspecific; not specifically directed against any particular infectious agent or tumor

• no clonal expansion of Ag specificity

• Same every time; no ‘memory’ as found in the adaptive immune system

• failure ==> adaptive immune response
Components of Innate Immunity

First line

1. Physical barriers
2. Chemical & biochemical barriers
3. Biological barriers (Normal flora)

Second line

A. Cells
   1. Natural killer
   2. Phagocytes
   3. Inflammatory cells

B. Soluble factors

C. Inflammatory barriers
## Anatomical /Physical/Mechanical Barriers

<table>
<thead>
<tr>
<th>System or Organ</th>
<th>Cell type</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Squamous epithelium</td>
<td>Physical barrier (intact skin) Desquamation</td>
</tr>
<tr>
<td>Mucous Membranes</td>
<td>Non-ciliated epithelium (<em>e.g.</em> GI tract)</td>
<td>Peristalsis</td>
</tr>
<tr>
<td></td>
<td>Ciliated epithelium, hairs (<em>e.g.</em> respiratory tract)</td>
<td>Mucociliary elevator, Coughing, sneezing</td>
</tr>
<tr>
<td></td>
<td>Epithelium (<em>e.g.</em> nasopharynx)</td>
<td>Flushing action of tears, saliva, mucus, urine; blinking of eye lids</td>
</tr>
</tbody>
</table>
## Biological Factors

<table>
<thead>
<tr>
<th>System or Organ</th>
<th>Component</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin and mucous membranes</td>
<td>Normal microflora</td>
<td>Antimicrobial substances</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Competition for nutrients and colonization</td>
</tr>
</tbody>
</table>
## Chemical Factors
(at surfaces and in body cavities)

<table>
<thead>
<tr>
<th>System or Organ</th>
<th>Component</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Sweat</td>
<td>Anti-microbial fatty acids, high salt conc.</td>
</tr>
<tr>
<td>Mucous Membranes</td>
<td>HCl (parietal cells)</td>
<td>Low pH</td>
</tr>
<tr>
<td></td>
<td>Tears and saliva</td>
<td>Lysozyme and phospholipase A</td>
</tr>
<tr>
<td></td>
<td>Defensins (respiratory &amp; GI tract)</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td></td>
<td>Sufactants (lung)</td>
<td>Opsonin</td>
</tr>
</tbody>
</table>
# Chemical Factors
*(Humoral Components)*

<table>
<thead>
<tr>
<th>Component</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement</td>
<td>Lysis of bacteria and some viruses, Opsonin</td>
</tr>
<tr>
<td></td>
<td>Increase in vascular permeability</td>
</tr>
<tr>
<td></td>
<td>Recruitment and activation of phagocytic cells</td>
</tr>
<tr>
<td>Coagulation system</td>
<td>Increase vascular permeability, Recruitment of phagocytic cells, β-lysin from platelets – a cationic detergent, antibacterial</td>
</tr>
<tr>
<td>Acute phase protein</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Breaks down bacterial cell walls</td>
</tr>
<tr>
<td>Lactoferrin and transferrin</td>
<td>Compete with bacteria for iron</td>
</tr>
<tr>
<td>Cytokines</td>
<td>Various effects</td>
</tr>
<tr>
<td>Interferon</td>
<td>Anti-viral protein</td>
</tr>
</tbody>
</table>
Pathways of complement activation

- CLASSICAL PATHWAY
  - antibody dependent

- LECTIN PATHWAY
  - antibody independent

- ALTERNATIVE PATHWAY

Activation of C3 and generation of C5 convertase

- activation of C5

LYTIC ATTACK PATHWAY
THE ACUTE PHASE PROTEINS

‘Acute phase proteins’ are a large group of plasma proteins whose concentration increases (or decreases) (by 25% or more) during inflammation (acute or chronic), such as injury and in disease states.

Proinflammatory cytokines stimulate hepatocytes in the liver to synthesize and secrete acute phase proteins.

Examples of acute phase proteins are CRP, MBP, haptoglobin, SAA, fibrinogen, $\alpha_1$-antitrypsin, and complement components C3 and C4.

C-reactive protein (CRP) binds to membrane phospholipids in microbial membranes.

Mannose binding protein (MBP) binds to mannose sugars found in many bacteria and fungi.

These functions as opsonins, soluble pattern-recognition receptors, activate the complement pathway or be involved in sequestration of essential nutrients.
Antimicrobial Peptides (AMPs)

• **Antimicrobial peptides** forms pores in the cytoplasmic membrane of a variety of bacteria causing leakage of cellular needs, e.g. lysozymes, lactoferrins, defensins, protegrins, granulocytes, etc.

• **Lysozyme**, in serum, mucus, plasma, tissue fluids and tears, breaks down the bacterial cell wall (peptidoglycan).

• **Beta-defensins** are short peptides found in blood plasma and mucous.

• **Transferrin & lactoferrin** competitively binds iron in blood, tissues and milk thereby preventing its availability to microorganisms.
Interferons (IFN)

- Interferons (IFNs) comprise a family of secreted α-helical cytokines induced in response to specific extracellular biomolecules of viruses or other pathogens through stimulation of Toll-like receptors (TLRs).
- Act in paracrine or autocrine modes for regulating innate and acquired immunity, resistance to viral infections, and normal and tumor cell survival and death.
- There are five types of human interferon: alpha, beta, gamma, delta and omega (α-IFN, β-IFN, γ-IFN, ξ-IFN, and ω-IFN, respectively).
- Virus infected cells produce IFN-α and IFN-β.
- Interferons are host-cell-specific, but not virus-specific.
- Gamma-IFN, also called as immune interferon, activates neutrophils, NK cells and macrophages.
- Interferons also result in resistance to viral replication; induce enzymes to degrade viral mRNA increased MHC I expression activate NK cells, T-cells and macrophages.
Interferons

Virus-infected cells (double-stranded RNA) → Interferon → Uninfected cells → Induces enzymes (inactive) capable of degrading mRNA → Uninfected cells become infected with a virus → Activation of enzymes → Degradation of viral and cellular mRNA → Blocks viral and cellular protein synthesis → Death of the infected cell
## Cellular Components

<table>
<thead>
<tr>
<th>Cell</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>Phagocytosis and intracellular killing</td>
</tr>
<tr>
<td></td>
<td>Inflammation and tissue damage</td>
</tr>
<tr>
<td>Macrophages</td>
<td>Phagocytosis and intracellular killing</td>
</tr>
<tr>
<td></td>
<td>Extracellular killing of infected or altered self targets</td>
</tr>
<tr>
<td></td>
<td>Tissue repair</td>
</tr>
<tr>
<td>NK cells</td>
<td>Killing of virus-infected and altered self targets</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Killing of certain parasites</td>
</tr>
</tbody>
</table>
Inflammation
Inflammation

• Inflammation is an attempt by the body to eliminate the noxious agent and restore and maintain homeostasis after injury to a tissue.

• The injury is often caused by invading organisms.

• It is the second line of defense.
Inflammation (contd.)

The principal effects of inflammation to a site of injury are:

• An increase in blood supply.

• An increase in vascular permeability to large serum molecules.

• Enhanced migration of leukocytes across the local vascular endothelium and in the direction of the site of inflammation.

• The inflammatory response is characterized by redness, heat, swelling and pain.
The Good Side of Inflammation

The inflammatory response to tissue damage is of great value by:

- isolating the damaged area
- mobilizing effector cells and molecules to the site, and
- in the late stages — promoting healing

Inflammation protects the body (innate immunity)
The Bad Side of Inflammation

Often the inflammatory response is out of proportion to the threat it is dealing with. The result can be more damaging to the body than the agent itself would have produced.

**Allergies and Autoimmune Diseases** are examples of inflammation in response to what should have been a harmless, or at least noninfectious, agent.
Phagocytosis
Phagocytosis

Phagocytosis is the ingestion of microorganisms or particulate matter by a cell.

Phagocytosis is performed by phagocytes—certain types of white blood cells or derivatives of them.

All phagocytes eat, digest and extrude...
Phagocytes and Their Relatives

- Monocyte
- Eosinophil
- Neutrophil
- Basophil
- Mast cell
- Dendritic cell
- Macrophage
Phagocytic Cells

Myeloid

• Neutrophils
  • Rapid phagocytosis, but cannot phagocytose repeatedly
  • Has granules which contain bactericidal enzymes
  • Short lived
  • NO ABILITY TO PRESENT ANTIGEN

• Eosinophils

Macrophage-Monocyte

• Basophils

• Slow but can phagocytose repeatedly.

• Contains bactericidal enzyme.

• Long lived

• Selected cells HAVE ability to present Ag.
Mechanism of Phagocytosis

1. Microbe adheres to phagocyte
2. Phagocyte forms pseudopods that eventually engulf the particle
3. Phagocytic vesicle is fused with a lysosome
4. Microbe in fused vesicle is killed and digested by lysosomal enzymes within the phagolysosome, leaving a residual body
5. Indigestible and residual material is removed by exocytosis
Chemotaxis & attachment

• Attraction by chemotactic substances (microbes, damaged tissues, complement components, vasoactive amines, etc)

• Attachment by receptors on surfaces of phagocytes.

Ingestion and phagosome formation

• Phagocytes’ produce pseudopodia surrounding organism forming phagosome

• Opsonins and co-factors enhance phagocytosis

Phagolysosome formation

• Fusion of phagosome with lysosomal granules of phagocyte take place by help of cytoskeleton followed by the release digestive and degradative enzymes
The Process of Phagocytosis - ingestion

Following attachment, polymerization and depolymerization of actin molecules send pseudopods out to engulf the bacterium.
The Process of Phagocytosis - phagosome formation

Following engulfment, the bacterium is placed in a vesicle called a phagosome.
Lysosomes move along the cytoskeleton and fuse with phagosomes to form phagolysosomes.
The lysosome, its digestive enzymes and microbicidal chemicals fuses with the phagosome containing the ingested bacteria to form a phagolysosome and the bacterium is killed.
Intra-cellular killing (two microbicidal routes)

- **Oxygen-dependent system** (powerful microbicidal agents)

  Oxygen converted to superoxide anion, hydrogen peroxide, activated oxygen and hydroxyl radicals.

- **Oxygen-independent system** (anaerobic conditions)

  Digestion and killing by lysozyme, lactoferrin, low pH, cationic proteins and hydrolytic and proteolytic enzymes
Pathways of Intracellular Killing

- Oxygen-dependent
- Oxygen-independent
  - Myeloperoxidase-independent
  - Myeloperoxidase-dependent
Respiratory Burst

Oxygen-dependent Myeloperoxidase-independent Reactions

\[
\begin{align*}
\text{Glucose} + \text{NADP}^+ & \rightarrow \text{Pentose-P} + \text{NADPH} \\
\text{G-6-P-dehydrogenase} & \\
\text{NADPH} + \text{O}_2 & \rightarrow \text{NADP}^+ + \text{O}_2^- \\
\text{Cytochrome } b_{558} & \\
\text{2O}_2^- + 2\text{H}^+ & \rightarrow \text{H}_2\text{O}_2 + \text{1O}_2 \\
\text{Superoxide dismutase} & \\
\text{2O}_2^- + \text{H}_2\text{O}_2 & \rightarrow \cdot\text{OH} + \text{OH}^- + \text{1O}_2
\end{align*}
\]

Toxic compounds – Superoxide anion (O2⁻), Hydrogen peroxide (H₂O₂), Singlet oxygen (¹O₂) and Hydroxyl radical (OH*)
**Respiratory Burst**

**Oxygen-dependent Myeloperoxidase-dependent Reactions**

\[
\begin{align*}
H_2O_2 + Cl^- & \xrightarrow{\text{myeloperoxidase}} OCl^- + H_2O \\
2OCl^- + H_2O & \rightarrow ^1O_2^- + Cl^- + H_2O
\end{align*}
\]

Toxic compounds
- Hypochlorous acid (OCl\(^-\))
- Singlet oxygen (^1O_2^-)
Respiratory Burst

Detoxification Reactions

\[ 2O_2^- + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2 \]

\[ 2H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2 \]
# Mediators of Oxygen-independent Killing in the Phagolysosome

<table>
<thead>
<tr>
<th>Effector Molecule</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cationic proteins (cathepsin)</td>
<td>Damage to microbial membranes</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Hydrolyses mucopeptides in the cell wall</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Deprives pathogens of iron</td>
</tr>
<tr>
<td>Hydrolytic enzymes (proteases)</td>
<td>Digests killed organisms</td>
</tr>
</tbody>
</table>
Some cytokines can also induce phagocytic cells, particularly macrophages, to produce nitric oxide (NO), which is toxic to microorganisms and malignant cells.
Interferon gamma \[ \rightarrow \] NO synthetase

Tumor necrosis factor \[ \rightarrow \] NO synthetase

\[ \text{O}_2 + \text{L-arginine} \rightarrow \text{NO} + \text{citrulline} \]

Cofactor: tetrahydrobiopterin
Nitric Oxide Dependent Killing

Nitric oxide also possess antiviral properties:

• inhibition of viral RNA synthesis
• inhibition of viral protein accumulation
• inhibition of virus release from infected cell
Extracellular Destruction of Bacteria by a Phagocyte

- If the phagocyte is overwhelmed with microorganisms, the phagocyte will empty the contents of its lysosomes by a process called degranulation in order to kill the microorganisms or cell extracellularly. These released lysosomal contents, however, also kill surrounding host cells and tissue. Most tissue destruction associated with infections is a result of this process.
FACTORS AFFECTING PHAGOCYTOSIS

**OPSONINS** “natural ketchup”
Proteins which coat the antigen to facilitate phagocytosis
  e.g. Antibodies
  Complement Components
  Certain Liver proteins

Generally both bacteria and cells that are suspended in body fluids have negative charges (*Zeta Potential*). Therefore they tend to repel each other. Opsonins, provide positive charges and coating with opsonins promotes phagocytosis.
<table>
<thead>
<tr>
<th>ANTIGEN</th>
<th>OPSONIN</th>
<th>EFFICIENCY OF PHAGOCYTOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Antibody</td>
<td>+++±</td>
</tr>
<tr>
<td></td>
<td>Complement</td>
<td>+++±</td>
</tr>
<tr>
<td></td>
<td>Antibody Plus Complement</td>
<td>+++±</td>
</tr>
</tbody>
</table>
Fever
Fever

- Activated macrophages and other leukocytes release proinflammatory cytokines such as TNF-alpha, IL-6, and IL-1.

- These cytokines stimulate the anterior hypothalamus of the brain to produce prostaglandins that lead to an increase in body temperature – fever.

- Fever increases the physiological temperature above the optimum growth temperature for many microorganisms.

- Fever leads to the production of heat shock proteins resulting in the production of inflammation-promoting cytokines.

- Fever elevates the temperature of the body increasing the rate of enzyme reactions, and speeding up metabolism within the body.
Fever and Immune Activation

LYMPHOCYTE ACTIVATION (T, B, NK)  
Alternate pathway of C'

MOTILITY OF NEUTROPHILS  
GROWTH OF BACTERIA

INCREASES NEUTROPHILS

Stimulates Fibroblast Proliferation

FEVER

IL-1, IL-6, TNF-α

BACTERIA

Mac

SLEEP

APPETITE

INCREASED AMINO ACIDS IN BLOOD

ACUTE PHASE PROTEINS

Stimulates Hepatocytes

LOWERS IRON, ZINC IN BLOOD

MUSCLE PROTEOLYSIS

INCREASES NEUTROPHILS

MOTILITY OF NEUTROPHILS

GROWTH OF BACTERIA

LYMPHOCYTE ACTIVATION (T, B, NK)  
Alternate pathway of C’
How innate Immunity Recognizes?
Pattern-Recognition Receptors

- Innate immunity recognize a few highly conserved structures present in many different microorganisms - the pathogen-associated molecular patterns (PAMPs) as well as danger signals released from damaged or dying (necrotic) cells (DAMPs).

- These PAMPs/DAMPs are recognized by pattern recognition receptors (PRRs), expressed on/in the innate immunity cells.

- PRRs can also recognize host molecules containing damage-associated molecular patterns (DAMPs), molecules that are often released from necrotic cells damaged by invading pathogens.

- These PRRs include Toll-like receptors (TLRs), nucleotide-binding domain (NOD) and leucine-rich repeat containing receptors (NLRs), and retinoic acid-inducible gene-I (RIG-) like receptors (RLRs), lectins, and scavenger receptors.
Innate immunity

Pathogen → PAMPs → PRR (e.g., TLR) → APC → T cell

Macrophage
PMN

STRANGERS

Cytokines/chemokines
Immune cell recruitment
Inflammation
Adaptive immunity
Tissue repair

DANGERS

Stressed
Healthy

DAMPs
DAMP receptor
PRR
PAMPs

- Lipopolysaccharide
- Peptidoglycan
- Lipoteichoic acids
- Mannose-rich glycans
- Flagellin
- Pilin
- Bacterial nucleic acid
- N-formylmethionine,
- Double-stranded RNA
- Lipoteichoic acids, glycolipids, and zymosan
- Phosphorylcholine and other lipids
PAMPs binding to PRRs on defense cells

The PRRs recognize approximately $10^3$ molecular patterns.
Innate immune responses encountered by microbes.

Microbes are detected by pattern recognition receptors (PRRs) expressed in innate immune cells, such as macrophages. The detection of microbes by the PRRs rapidly activates signalling cascades and generates inflammatory responses. Microbial encounter also leads to maturation of macrophages and dendritic cells into antigen presenting cells. PAMP, pathogen-associated molecular pattern; TCR, T-cell receptor.
<table>
<thead>
<tr>
<th>PAMP</th>
<th>PRR</th>
<th>Biological Consequence of Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial cell wall components</td>
<td>Complement</td>
<td>Opsonization; Complement activation</td>
</tr>
<tr>
<td>Mannose-containing carbohydrates</td>
<td>Mannose-binding protein</td>
<td>Opsonization; Complement activation</td>
</tr>
<tr>
<td>Lipoproteins of Gram positive bacteria, yeast cell wall components</td>
<td>TLR-2 (Toll-like receptor 2)</td>
<td>Macrophage activation; Secretion of inflammatory cytokines</td>
</tr>
<tr>
<td>Double stranded RNA</td>
<td>TLR-3</td>
<td>Production of interferon (antiviral)</td>
</tr>
<tr>
<td>LPS (lipopolysaccharide of Gram –ve bacteria)</td>
<td>TLR-4</td>
<td>Macrophage activation; Secretion of inflammatory cytokines</td>
</tr>
<tr>
<td>Flagellin (bacterial flagella)</td>
<td>TLR-5</td>
<td>Macrophage activation; Secretion of inflammatory cytokines</td>
</tr>
</tbody>
</table>
DAMPs

• Damage-associated molecular patterns (DAMPs) are endogenous danger molecules that are released upon cellular stress or tissue injury from damaged or dying cells and activate the innate immune system by interacting with pattern recognition receptors (PRRs).
• DAMPs activate the innate immune system by inducing potent inflammatory responses during non-infectious inflammation.
• These DAMPs are recognized by macrophages, and inflammatory responses are triggered by different pathways, including TLRs and inflammasomes.
• DAMPs can originate from different sources and include:
  – extracellular proteins, e.g. biglycan and tenascin C,
  – intracellular proteins, e.g. high-mobility group box 1 (HMGB1), histones, S100 proteins, heat-shock proteins (HSPs), and
  – plasma proteins, e.g. fibrinogen, Gc-globulin, and serum amyloid A (SAA).
Induction of immune response by DAMPs
Toll-like receptors (TLRs) - 1

- Play a major role in innate immunity and the induction of adaptive immunity.
- Different combinations of TLRs appear in different cell types and seem to appear in pairs; 13 recognized so far.
- Different TLRs directly or indirectly bind different microbial molecules.
- **TLRs are found both on the surface and within the phagolysosomes of phagocytes.**
- Surface TLRs recognize molecules on the surface of microbes such as cell wall components.
- Internal TLRs recognize microbial molecules released upon phagocytosis of the microbe.
Consequences of TLR Signal Transduction

NF$_{κ}$B Activation → Transcriptional Activation

Synthesis of:
- ROIs
- Anti-microbial peptides
- Cytokines
- Chemokines
- Adhesion molecules
- Acute phase proteins