Nonspecific Defense Mechanisms
Introduction

- Most microbes reproduce rapidly and would quickly overwhelm the body in the time it takes to develop an adaptive immune response.

- Innate immunity responds rapidly to infection and provide protection while antigen-specific lymphocytes prepare to act.
Characteristics of Nonspecific immune mechanisms

- Innate
- Prepared to react at once
- Steady during the reaction
- Same intensity
- Same during the life
- Same type of reactions against any invader
- Without memory
- Always as for the first time
Innate Immunity

- Innate immunity is germ line encoded (individuals are born with it ready to go); it has made the self/nonself discrimination on an evolutionary time-scale.

- It uses few receptors that recognize features common to many microorganisms.

- Therefore, parts of it are always active or can be activated quickly.

- Innate immunity comprises the first and second lines of defense.

- Without innate immunity nearly every microorganism would be pathogenic.
Innate immunity (immediate: 0–4 hours)

Early induced response (early: 4–96 hours)

Adaptive immune response (late: >96 hours)

Infection → Recognition by preformed, nonspecific effectors → Removal of infectious agent

Infection → Recruitment of effector cells → Recognition, activation of effector cells → Removal of infectious agent

Infection → Transport of antigen to lymphoid organs → Recognition by naive B and T cells → Clonal expansion and differentiation to effector cells → Removal of infectious agent

Fig 2.1 © 2001 Garland Science
- Activation of phagocytic cells and soluble molecules leading to inflammation

- Containment and destruction of infectious agent

- Participation in the induction of adaptive immune responses.
Barriers of the Innate Immune System

- Initial protection is achieved by barriers that guard body’s interface with the environment.

- These include the skin and mucous membranes of the gastrointestinal, respiratory, and genitourinary systems.

- The sebaceous glands of the dermal layer secrete sebum that contains lactic acid, and a variety of fatty acids whose low pH gives them microbicidal activity.

- Skin secretions and mucosal surfaces contain microbicidal molecules such as β-defensins.
- Skin flora prevents colonization by pathogenic organisms

- Mucosal surfaces are covered with thick secretions that may be acidic, and contain enzymes (lysozyme), and microbicidal molecules (α defensin, cryptidin)

- Hair and cilia have entrapping activity that is completed by coughing and sneezing

- Lacrimation, salivation, urination, and peristaltic movements discharge microorganisms

- Normal flora protects from pathogenic organisms by various mechanisms.
<table>
<thead>
<tr>
<th><strong>Intrinsic epithelial barriers to infection</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mechanical</strong></td>
</tr>
<tr>
<td>- Epithelial cells joined by tight junctions</td>
</tr>
<tr>
<td>- Longitudinal flow of air or fluid across epithelium</td>
</tr>
<tr>
<td>- Movement of mucus by cilia</td>
</tr>
<tr>
<td><strong>Chemical</strong></td>
</tr>
<tr>
<td>- Fatty acids (skin)</td>
</tr>
<tr>
<td>- Enzymes: lysozyme (saliva, sweat, tears), pepsin (gut)</td>
</tr>
<tr>
<td>- Low pH (stomach)</td>
</tr>
<tr>
<td>- Antibacterial peptides; defensins (skin, gut), cryptidins (intestine)</td>
</tr>
<tr>
<td><strong>Microbiological</strong></td>
</tr>
<tr>
<td>- Normal flora compete for nutrients and attachment to epithelium and can produce antibacterial substances</td>
</tr>
</tbody>
</table>
Receptors of innate immunity

- Phagocytic receptors (PRR)

- Chemotactic receptors: Induce production/activation of other signaling molecules (e.g., cause cytokine production and secretion)

- MB lectin binds patterns of mannan

- Scavenger receptor binds certain charged particles (anionic polymers)

- LPS-binding protein (CD14) binds LPS
Recognition by Toll-Like Receptors

- Innate immunity utilizes a limited number of germline-encoded receptors that recognize conserved molecules.

- Pattern recognition receptors (PRRs) on host cells and certain soluble molecules can recognize pathogen-associated molecular patterns (PAMPs).

- Toll-like receptors (TLRs): 13 different TLRs have been identified in humans that are distributed on different cells.

- Binding of a TLR triggers cells that participate in some aspects of inflammation like macrophages, dendritic cells, mast cells, and some epithelial cells.
### Toll-Like Receptors and their Ligands

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand (PAMPs)</th>
<th>Origin of Ligand</th>
</tr>
</thead>
</table>
| TLR1     | Triacyl lipopeptides  
Soluble factors | Bacteria and Mycobacteria  
*Neisseria meningitidis* |
| TLR2     | Heat Shock protein 70  
Peptidoglycan  
Lipoprotein/lipopptides  
HCV core and nonstructural 3 protein | Host  
Gram-positive bacteria  
Various pathogens  
Hepatitis C Virus |
| TLR3     | Double-stranded RNA | Viruses |
| TLR4     | Lipopolysaccharides  
Envelope protein  
Taxol | Gram-negative bacteria  
Mouse mammary-tumor virus  
Plants |
| TLR5     | Flagellin | Bacteria |
| TLR6     | Zymosan  
Lipoteichoic acid  
Diacyl lipopolipetides | Fungi  
Gram-positive bacteria  
Mycoplasma |
| TLR7     | Single-stranded RNA (ssRNA)  
Imidazoquinoline | Viruses  
Synthetic compounds |
| TLR8     | Single-stranded RNA (ssRNA)  
Imidazoquinoline | Viruses  
Synthetic compounds |
| TLR9     | CpG-containing DNA | Bacteria, Malaria and Viruses |
| TLR10    | Not determined | Not Determined |
| TLR11    | Profilin-like molecule | *Toxoplasma gondii* |
TLR Signaling Pathways

Cell membrane

TLR2/TLR1  TLR2/TLR6  TLR4  TLR3

MAL  MyD88  MAL  MyD88  TRIF  TRAM  TRIF

NF-κB  IRF3  IRF7

Interferon Pathway

Inflammatory Cytokines

Endosome
Toll-like receptor pathway
Involvement of TLR in Linking Innate Immunity to Adaptive Immunity
The macrophage expresses receptors for many bacterial constituents

- mannose receptor
- LPS receptor (CD14)
- CD11b/CD18
- scavenger receptor

Bacteria binding to macrophage receptors initiate the release of cytokines and small lipid mediators of inflammation

- lipid mediators
- cytokines

Macrophages engulf and digest bacteria to which they bind

- phagosome
- lysosome
- phagolysosome

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Phagocytosis

1. Recognition and Attachment
   - Microbes bind to phagocyte receptors
     - Mac-1 integrin
     - Scavenger receptor
     - Mannose receptor

2. Engulfment
   - Phagocyte membrane zips up around microbe
   - Microbe ingested in phagosome

3. Killing and Degradation
   - Killing of microbes by ROIs and NO
     - Arginine
     - NO
     - ROI
     - O₂
     - Phagocyte oxidase
   - Killing of microbes by lysosomal enzymes in phagolysosome
   - Fusion of phagosome with lysosome
Neutrophils

- They are the first cell-type to arrive at the site of acute inflammation.

- In the tissue they have a life span of a few days.

- Contain lytic enzymes and bactericidal substances in granules.

- Contents of the granules are also secreted extracellulary during phagocytosis.
- Granules contain the enzyme, myeloperoxidase (MPO), which in the presence of halide ion can convert \( \text{H}_2\text{O}_2 \) into hypochlorite, which is a potent antimicrobial substance.

- Kill via oxygen-dependent as well as independent pathways.

- Have a larger respiratory burst than macrophages and are more efficient in killing microorganisms.
<table>
<thead>
<tr>
<th>Name</th>
<th>Tissue distribution</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adhesion Molecules Direct Trafficking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Selectins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-selectin (PADGEM, CD62P)</td>
<td>Activated endothelium and platelets</td>
<td>PSGL-1, sialyl-Lewisx</td>
</tr>
<tr>
<td>E-selectin (ELAM-1, CD62E)</td>
<td>Activated endothelium</td>
<td>Sialyl-Lewisx</td>
</tr>
<tr>
<td><strong>Integrins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha_1\beta_2) (LFA-1, CD11a/CD18)</td>
<td>Monocytes, T cells, macrophages, neutrophils, dendritic cells</td>
<td>ICAMs</td>
</tr>
<tr>
<td>(\alpha_M\beta_2) (Mac-1, CR3, CD11b/CD18)</td>
<td>Neutrophils, monocytes, macrophages</td>
<td>ICAM-1, iC3b, fibrinogen</td>
</tr>
<tr>
<td>(\alpha_x\beta_2) (CR4, (\alpha_{5}\beta_1) (VLA-5, CD49d/CD29)</td>
<td>Dendritic cells, macrophages, neutrophils</td>
<td>iC3b</td>
</tr>
<tr>
<td><strong>Immunoglobulin superfamily</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICAM-1 (CD54)</td>
<td>Activated endothelium</td>
<td>LFA-1, Mac1</td>
</tr>
<tr>
<td>ICAM-2 (CD102)</td>
<td>Resting endothelium, dendritic cells</td>
<td>LFA-1</td>
</tr>
<tr>
<td>VCAM-1 (CD106)</td>
<td>Activated endothelium</td>
<td>VLA-4</td>
</tr>
<tr>
<td>PECAM (CD31)</td>
<td>Activated leukocytes, endothelial cell–cell junctions</td>
<td>CD31</td>
</tr>
</tbody>
</table>

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Selectin-mediated adhesion to leukocyte sialyl-Lewis\textsuperscript{x} is weak, and allows leukocytes to roll along the vascular endothelial surface.

**Rolling adhesion**

**Tight binding**

**Diapedesis**

**Migration**

- IL-8 receptor
- LFA-1 (\(\alpha_L:\beta_2\))
- ICAM-1
- E-selectin
- CD31
- Chemokine (IL-8)
Macrophages

- Ingest bacteria, viruses, dead cells, and dust

- Resident or circulating cells in the blood, lymph and extracellular fluid

- They are attracted to the site of infection by chemicals released by dying cells

- After ingesting a foreign invader, they present antigens of it to T and B lymphocytes
Oxygen-dependent killing during phagocytosis by respiratory burst occurs in activated macrophages.

This results in the activation of a membrane-bound oxidase (NADPH oxidase) which catalyzes the reduction of oxygen to various oxygen radicals that are toxic to the ingested microbe.

Macrophages activated with bacterial cell wall components such as LPS express high level of the enzyme Nitric Oxide Synthetase (NOS), that generates nitric oxide which has antimicrobial activity.
Oxygen-independent killing mechanisms

- Hydrolytic enzymes
- Lysozyme
- Antimicrobial and cytotoxic peptides such as defensins
Agents produced by phagocytes (Macrophages and/or Neutrophils) upon bacterial stimulation

<table>
<thead>
<tr>
<th>Class of mechanism</th>
<th>Specific products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidification</td>
<td>pH = 3.5 – 4.0, bacteriostatic or bactericidal</td>
</tr>
<tr>
<td>Toxic oxygen-derived products</td>
<td>Superoxide $\text{O}_2^-$, hydrogen peroxide $\text{H}_2\text{O}_2$, singlet oxygen $^1\text{O}_2^<em>$, hydroxyl radical $\text{OH}^</em>$, hypohalite $\text{OCl}^-$</td>
</tr>
<tr>
<td>Toxic nitrogen oxides</td>
<td>Nitric oxide NO</td>
</tr>
<tr>
<td>Antimicrobial peptides</td>
<td>Defensins and cationic proteins</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Lysozyme — dissolves cell walls of some gram-positive bacteria. Acid hydrolases—further digest bacteria</td>
</tr>
<tr>
<td>Competitors</td>
<td>Lactoferrin (binds Fe) and vitamin $\text{B}_{12}$-binding protein</td>
</tr>
</tbody>
</table>
Activated Phagocytes: Macrophages and Dendritic Cells

- Increase in size and in the rate of production of degradative enzymes and microbicidal molecules.

- The rate of killing increases and they secrete soluble mediators (IL-1, IL-6, IL-8, IL-12, TNFα).

- Attraction and activation of other cells involved in innate immunity.
The macrophages’ effects on endothelial cells, which largely control inflammation by controlling the flow of cells and fluids out of the post-capillary venules, result from release of prostaglandins, leukotrienes and cytokines such as IL-1 and tumor necrosis factor-α (TNFα).

Blood coagulation stops bleeding and prevents pathogens from entering the circulation.
The same compounds are involved in adaptive immune responses (T\textsubscript{H}1) but probably more...
Systemic affects of Macrophage-produced cytokines

- **Liver**: Acute-phase proteins (C-reactive protein, mannan-binding lectin), Activation of complement Opsonization
- **Bone marrow endothelium**: Neutrophil mobilization, Phagocytosis
- **Hypothalamus**: Increased body temperature, Decreased viral and bacterial replication, Increased antigen processing, Increased specific immune response
- **Fat, muscle**: Protein and energy mobilization to allow increased body temperature
- **Dendritic cells**: TNF-\(\alpha\) stimulates migration to lymph nodes and maturation, Initiation of adaptive immune response

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The Complement System

- Discovered as a heat-labile antibacterial substance in immune serum
- Two components are needed for bacterial inactivation: a heat-stable immune component (antibody) and a heat-labile non immune component (complement).
- The complement system is comprised of many proteins that react with each other and with other compounds to
  1. Opsonize
  2. Kill cells
  3. Induce inflammation
Natural killer cells (NK cells)

- Instead of attacking the invaders, they attack the body’s own cells that have become infected by viruses.

- They also attack potential cancer cells, often before they form tumors.

- They bind to cells using an antibody “bridge”, then kill it by secreting a chemical (perforin) that makes holes in the cell membrane of the target cell.

- With enough holes, the cell will die, because water rushing inside the cell will induce osmotic swelling, and an influx of calcium may trigger apoptosis.
Recognition by Natural Killer Cells

- Use a recognition mechanism that detects alteration in host cells that are induced by infection or transformation.

- They recognize antibody coated cells through a low affinity receptor (CD16) and they lyse by ADCC.

- They express CR3 and CR4 that recognize and bind to membrane bound C3b.

- Certain NK cells recognize “stress-induced proteins” like heat shock protein and adhesion molecules.
NK cells distinguish normal from infected or transformed cells by monitoring the amount of surface MHC class I.

NK cells bear a killer activation receptor (KAR) called NKG2D that recognizes and binds certain molecules (MICAs and MICBfs) that appear on cells undergoing stress which provides a kill signal.

However, once contact is made with stressed target cells, NK cells use a second set of receptors, the killer inhibitory receptors (KIRs).
Target cell is examined for the expression and levels of self MHC class I, and if the KIRs locate and bind sufficient MHC class I the kill signal is overridden to prevent cell killing.

NK cell cytotoxic activity is augmented in the presence of type 2 interferon and IL-12 produced by phagocytic cells.
NK-cell Receptors and Killing

**Activating receptor** (e.g., NKG2D)
- Ligands for activating receptor: stress-induced proteins, viral proteins

**Inhibitory receptor** (KIRs, CD94-NKG2A,B)
- Ligand for inhibitory receptor: self class I MHC molecules

**Normal cell**
- Increased expression
- Viral infection, malignant transformation of normal cell
- Decreased expression of class I MHC molecules
- Inhibitory receptor not engaged
- ↑ ligand; activating receptors engaged

**Cell killing**
MHC class I on normal cells is recognized by killer inhibitory receptors (KIRs) or by lectinlike CD94:NKG2 heterodimers on NK cells, which inhibit signals from activating receptors.

NK cell does not kill the normal cell.

'Altered' or absent MHC class I cannot stimulate a negative signal. NK cell is triggered by signals from activating receptors.

Activated NK cell releases granule contents, inducing apoptosis in target cell.

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Certain defense mechanisms seem to fall between innate and adaptive immunity.

They use an immunoglobulin or a TCR to bind antigens but they have limited repertoires that appears to be germline encoded.

These include:
1. A subset of T cells called γδ T cells (mostly in skin and near epithelial surfaces)
2. CD5+ B cells (i.e., B-1 B cells) (mostly in the peritoneum)
3. Natural antibodies
<table>
<thead>
<tr>
<th>Class</th>
<th>Chemokine</th>
<th>Produced by</th>
<th>Receptors</th>
<th>Cells attracted</th>
<th>Major effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>MIP-1α</td>
<td>Monocytes T cells Mast cells Fibroblasts</td>
<td>CCR1, 3, 5</td>
<td>Monocytes NK and T cells Basophils Dendritic cells</td>
<td>Competes with HIV-1 Antiviral defense Promotes TH1 immunity</td>
</tr>
<tr>
<td></td>
<td>MIP-1β</td>
<td>Monocytes Macrophages Neutrophils Endothelium</td>
<td>CCR1, 3, 5</td>
<td>Monocytes NK and T cells Dendritic cells</td>
<td>Competes with HIV-1</td>
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<tr>
<td></td>
<td>MCP-1</td>
<td>Monocytes Macrophages Fibroblasts Keratinocytes</td>
<td>CCR2B</td>
<td>Monocytes NK and T cells Basophils Dendritic cells</td>
<td>Activates macrophages Basophil histamine release Promotes TH2 immunity</td>
</tr>
<tr>
<td></td>
<td>RANTES</td>
<td>T cells Endothelium Platelets</td>
<td>CCR1, 3, 5</td>
<td>Monocytes NK and T cells Basophils Eosinophils Dendritic cells</td>
<td>Degranulates basophils Activates T cells Chronic inflammation</td>
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<tr>
<td></td>
<td>Eotaxin</td>
<td>Endothelium Monocytes Epithelium T cells</td>
<td>CCR3</td>
<td>Eosinophils Monocytes T cells</td>
<td>Role in allergy</td>
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<tr>
<td></td>
<td>DC-CK</td>
<td>Dendritic cells</td>
<td>?</td>
<td>Naive T cells</td>
<td>Role in activating naive T cells</td>
</tr>
<tr>
<td>Class</td>
<td>Chemokine</td>
<td>Produced by</td>
<td>Receptors</td>
<td>Cells attracted</td>
<td>Major effects</td>
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<tr>
<td>CXC</td>
<td>IL-8</td>
<td>Monocytes, Macrophages, Fibroblasts, Keratinocytes, Endothelial cells</td>
<td>CXCR1, CXCR2</td>
<td>Neutrophils, Naive T cells</td>
<td>Mobilizes, activates and degranulates neutrophils, Angiogenesis</td>
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<td></td>
<td>PBP β-TG NAP-2</td>
<td>Platelets</td>
<td>CXCR2</td>
<td>Neutrophils</td>
<td>Activates neutrophils, Clot resorption, Angiogenesis</td>
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<td>GROα, β, γ</td>
<td>Monocytes, Fibroblasts, Endothelium</td>
<td>CXCR2</td>
<td>Neutrophils, Naive T cells, Fibroblasts</td>
<td>Activates neutrophils, Fibroplasia, Angiogenesis</td>
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<td>IP-10</td>
<td>Keratinocytes, Monocytes, T cells, Fibroblasts, Endothelium</td>
<td>CXCR3</td>
<td>Resting T cells, NK cells, Monocytes</td>
<td>Immunostimulant, Antiangiogenic, Promotes TH1 immunity</td>
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<td></td>
<td>SDF-1</td>
<td>Stromal cells</td>
<td>CXCR4</td>
<td>Naive T cells, Progenitor (CD34⁺) B cells</td>
<td>B-cell development, Lymphocyte homing, Competes with HIV-1</td>
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<tr>
<td></td>
<td>BLC</td>
<td>Stromal cells</td>
<td>CXCR5</td>
<td>B cells</td>
<td>Lymphocyte homing</td>
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</table>
Chemokines, linking innate and adaptive immunity