CB12:
Host immune system: Innate immunity

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Objectives: The students can explain

• the role of innate immunity against infection.

• how physical and chemical barriers protect the body from infection.

• phagocytosis can defend against pathogens.

• complement can defend against pathogens.

• the linkage between innate and adaptive immunity.
Features of innate immune recognition

• Antigen-nonspecific defense mechanisms
• Immediate/within hours
• The immunity one is born with
• Initial response by the body to eliminate microbes and prevent infection
• Recognize a few highly conserved structures present in many different microorganisms (known as PAMPs)
• Innate immunity involves

1. Anatomical & chemical barriers, mechanical removal and normal flora

2. Pattern-recognition receptors, antigen-nonspecific antimicrobial body molecules and cytokines

3. The complement system

4. Cells involved in body defense
   - 4.1. Phagocytic cells: neutrophils, monocytes, macrophages
   - 4.2. Cells that release inflammatory mediators: basophils, mast cells and eosinophils
   - 4.3. Natural killer cells (NK cells)
   - 4.4. Antigen presenting cells

5. Phagocytosis

6. Inflammation

7. Fever
1. Anatomical & chemical barriers, mechanical removal and normal flora
Epithelial barriers

Epithelia at the portals of entry of microbes provide physical barriers, produce antimicrobial substances, and harbor intraepithelial lymphocytes that are believed to kill microbes and infected cells.

(Doan, T. Immunology, 2008)

Skin

- Dry
- Acidic
- <37°C
- normal flora
- Lysozyme

(Bauman, RW. Microbiology, 2005)
Chemical factors: Antigen-nonspecific antimicrobial body chemical

- **Hydrochloric acids**
- **Lysozyme**: tears, mucous, saliva, plasma, tissue fluid
  - Break down peptidoglycan
- **Human beta defensins**: in blood plasma & mucous
  - Form pores in CM, causing leakage
- **Lactic acid and fatty acids**
  - Inhibit mo. on the skin
- **Lactoferrin**: in secretions, plasma, tissue fluid
  - Trap iron

Lactoferrin alters the permeability of the lipopolysaccharide layer giving lysozyme access to the peptidoglycan layer.

Lactoferrin in secretions, plasma, tissue fluid.

Formation of multimeric pores within bacterial cell membrane.

Lactoferrin.

Iron will be mine!

Iron is my middle name!
Defense mechanisms of mucous membrane

- Goblet cells secrete mucus
- Specialized cells produce acids
- A variety of cells in the salivary glands and GI tract secrete digestive enzymes
- Specialized cells secrete microcidal molecules
• Biological barriers: commensal microbes

- **Scalp**
  - As for skin

- **Oral cavity**
  - Viridans streptococci
  - Anaerobic Gram-positive bacilli (including Actinomyces spp.)
  - Anaerobic Gram-negative bacilli
    - *Prevotella* spp.
    - *Fusobacterium* spp.
    - *Candida* spp.

- **Skin**
  - Coagulase-negative staphylococci
  - *Staph. aureus*
  - *Corynebacterium* spp.
  - *Propionibacterium* spp.
  - *Malassezia* spp.

- **Hands**
  - Resident: as for skin
  - Transient: skin flora (including meticillin-resistant and other *Staph. aureus*), bowel flora (including *Clostridium difficile*, *Candida* spp. and *Enterobacteriaceae*)

- **Vagina**
  - *Lactobacillus* spp.
  - *Staph. aureus*
  - *Candida* spp.
  - *Enterobacteriaceae*

- **Perineum**
  - As for skin
  - As for large bowel

- **Nares**
  - *Staph. aureus*
  - Coagulase-negative staphylococci

- **Pharynx**
  - *Haemophilus* spp.
  - *Moraxella catarrhalis*
  - *Nasal. anaerobes (including N. meningitidis)*
  - *Staph. aureus*
  - *Strep. pneumoniae*
  - *Strep. pyogenes (group A)*
  - *Viridans streptococci*

- **Small bowel**
  - Distally, progressively increasing numbers of large bowel bacteria
  - *Candida* spp.

- **Large bowel**
  - *Enterobacteriaceae*
  - *Escherichia coli*
  - *Klebsiella* spp.
  - *Enterobacter* spp.
  - *Proteus* spp.
  - *Enterococci*
  - *E. faecalis*
  - *E. faecium*
  - *Miller group streptococci*
  - *Strep. anginosus*
  - *Strep. intermedius*
  - *Strep. constellatus*
  - Anaerobic Gram-positive bacilli
  - *Clostridium* spp.
  - Anaerobic Gram-negative bacilli
  - *Bacteroides* spp.
  - *Prevotella* spp.
  - *Candida* spp.

- **Bacterial antagonism by normal flora**
  - Producing metabolic products
    - Fatty acids, bacteriocins etc. inhibit the growth of many pathogens
  - Adhering to target host cells, preventing pathogens from colonizing
  - Depleting nutrients
  - Nonspecifically stimulating the immune system
• Mechanical removal
  • Mucus and cilia
  • The cough and sneeze reflex
  • Vomiting and diarrhea
  • The physical flushing action of body fluids

(Bauman, RW. Microbiology, 2005)
2. Pattern-recognition receptors

Can recognize $\sim10^3$ molecular pattern

Recognize unique molecules of microorganisms that **are not** associate with human cells (PAMPs)

- LPS
- Peptidoglycan
- Lipoteichoic acids
- Mannose-rich glycans
- Flagellin
- Pillin
- Bacterial nucleic acid (high frequency of unmethylated cytosine-guanine dinucleotide sequences)
- N-formylmethionine
- dsRNA
- Lipoteichoic acid, glycolipid, zymosan
- Phosphorlycholine and other lipids
Two functional of pattern-recognition receptors

1. Endocytic pattern-recognition receptors
   - Found on surface of phagocytes
   - Promote the attachment of microorganisms to phagocytes, engulf and destroy

Exp: mannose receptors ( = C-type lectin)
   - bind to mannose and fucose of microbial glycoproteins and glycolipids

: scavenger receptors:
   - bind to CW e.g. LPS, peptidoglycan, teichoic acids

: N-formyl Met-Leu-Phe receptors
   - on neutrophils, macrophages
   - facilitate bacterial engulfment
2. Signaling pattern-recognition receptors

• Biding: LPS, peptidoglycan, teichoic acids, flagellin, pillin, bact. DNA, lipoteichoic acids, glycolipids, zymonsan, dsRNA etc.
• Promotes the synthesis and secretion of cytokines
• Exp.
  • TLR
  • NLRs (Nod-Like Receptors)
  • RLRs (Rig-Like Receptors)
NLRs (Nod-Like Receptors)

- Cytoplasmic molecules
- = intra cellular sensor for bacterial infections
- Nod = nucleotide-binding oligomerization domain
  - Activate TNF, IL-1β, IL-8, IFNα/β production
  - NOD1, NOD2
  - Mutated NOD2 can cause Crohn’s disease

RLRs (RIG-like receptors)

- Cytoplasmic receptors
- Including
  - retinoic acid inducible gene-I (RIG-I)
  - Melanoma differentiation-associated gene 5 (MDA5)
- Involve in interferon type 1 (IFNα and IFNβ) production to control viral infection
Biologic actions of type I interferons (IFN-α, IFN-β)
Cytokines

- Low MW, soluble proteins
- Functions as “chemical messengers”
- Promote inflammation by recruit WBC
- Regulate innate and adaptive immunity

Properties of cytokines
Cytokines of Innate Immunity

- Interleukins – produced and act on leukocytes
- Chemokines – direct cell movement
- TNF, IL-1 - active vessels and recruit Neutrophils
- IL-1 – Fever
- IL-6 – Acute phase response

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Principal cell source(s)</th>
<th>Principal cellular targets and biologic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin (IL-1)</td>
<td>Macrophages, endothelial cells, some epithelial cells</td>
<td>Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute phase proteins T cells: Th17 differentiation</td>
</tr>
<tr>
<td>Chemokines</td>
<td>Macrophages, dendritic cells, endothelial cells, T lymphocytes, fibroblasts, platelets</td>
<td>Leukocytes: Increased integrin affinity, chemotaxis, activation</td>
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<tr>
<td>Interleukin-12 (IL-12)</td>
<td>Dendritic cells, macrophages,</td>
<td>NK cells and T cells: IFN-γ production, increased cytokotoxic activity T cells: Th1 differentiation</td>
</tr>
<tr>
<td>Interferon-γ (IFN-γ)</td>
<td>NK cells, T lymphocytes</td>
<td>Activation of macrophages Stimulation of some antibody responses</td>
</tr>
<tr>
<td>Type I IFNs (IFN-α, IFN-β)</td>
<td>IFN-α: dendritic cells, macrophages IFN-β: fibroblasts</td>
<td>All cells: anti-viral state, increased class I MHC expression NK cells: activation</td>
</tr>
<tr>
<td>Interleukin-10 (IL-10)</td>
<td>Macrophages, dendritic cells, T cells</td>
<td>Macrophages, dendritic cells: inhibition of IL-12 production, reduced expression of costimulators and class II MHC molecules</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
<td>Macrophages, endothelial cells, T cells</td>
<td>Liver: synthesis of acute phase proteins B cells: proliferation of antibody-producing cells T cells: Th17 differentiation</td>
</tr>
<tr>
<td>Interleukin-15 (IL-15)</td>
<td>Macrophages, others</td>
<td>NK cells: proliferation T cells: proliferation</td>
</tr>
<tr>
<td>Interleukin-18 (IL-18)</td>
<td>Macrophages</td>
<td>NK cells and T cells: IFN-γ production</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Many cell types</td>
<td>Inhibition of inflammation T cells: differentiation of Th17, regulatory T cells</td>
</tr>
</tbody>
</table>
3. Complement systems

The classical complement pathway
- Activated by antigen-antibody complex

The alternative complement pathway
- Activated by C3b binding to microorganism’s surfaces and to antibody molecules

The lectin pathway
- Activated by the interaction of microorganism’s CHO with mannose-binding proteins
Alternative Pathway

1. Binding of complement proteins to microbial cell surface or antibody

2. Formation of C3 convertase

3. Cleavage of C3

4. Formation of C5 convertase

Classical Pathway

1. IgG antibody

2. C3 convertase

3. C3 convertase

4. C5 convertase

Lectin Pathway

1. Mannose

2. MASP1

3. MASP2

4. Mannose binding lectin

5. C3 convertase

6. C5 convertase

7. C5 convertase
Functions of complement

A. Opsonization and phagocytosis
- Binding of C3b (or C4b) to microbe (opsonization)
- Recognition of bound C3b by phagocyte C3b receptor

B. Stimulation of inflammatory reactions
- Binding of C3b to microbe, release of C3a; proteolysis of C5, releasing C5a
- Recruitment and activation of leukocytes by C5a, C3a
- Destruction of microbes by leukocytes

C. Complement-mediated cytolysis
- Binding of C3b to bacteria, activation of late components of complement
- Formation of the membrane attack complex (MAC)
- Osmotic lysis of bacteria
Cells of the immune system
4. Cells involved in body defense in innate immunity

4.1. Phagocytic cells: neutrophils, monocytes, macrophages, dendritic cells

4.2. Cells that release inflammatory mediators: basophils, mast cells and eosinophils

4.3. Natural killer cells (NK cells)

4.4. Antigen presenting cells
Maturation of mononuclear phagocytes
Phagocytic cells

1. Neutrophils

- = “polymorphonuclear neutrophils (PMN)”
- 1-2 days (7-10 hr in peripheral blood)
- Stem cells \( \xrightarrow{\text{granulocyte-colony stimulating factors (G-CSF)}} \) PMN, \( > 1 \times 10^{11} \) cells/day
- High ability to adhere and motile
  - Chemotactic agents: C5a, bacterial products, IL-8, leukotriene B4, IFN-γ
- major for acute inflammatory responses to bacterial infections
- The first cells recruited to acute inflammatory sites
(Abbas et al. Cellular and Molecular Immunology, 2012)
2. Monocytes

- ~10% of circulating WBC
- Circulate in blood and mature
- Phagocytosis
- Respond to chemokines to infection sites

1-2 days

Macrophages
3. Macrophages

- Derived from monocytes
  - Enter tissues and mature
    - liver = Kupffer cells
    - brain = microglia
    - kidney = mesangial cells
    - bone = osteoclasts
  - Tissues = macrophages

Reside for up to several months as macrophage
Figure 1. Functional heterogeneity in resident and recruited macrophages.
Th1 cells, NK cells, APC → IFN-γ → M1 Macrophages → Enhanced phagocytosis, Antigen presentation, Proinflammatory cytokines, ROI/RNI

Th2 cells, Granulocytes → IL-4, IL-13 → M2 Macrophages → Wound healing, Anti-parasitic, Anti-allergic

Treg cells → IL-10 → Regulatory Macrophages → Immune regulation
4. Dendritic cells

- Linking innate and adaptive immunity
- Distribute in lymphoid tissues, skin, gastrointestinal and respiratory tracts
- Antigen presenting cells to helper T & cytotoxic T lymphocytes
- Very effective in presenting Ag to virgin CD4+ T cells (naïve T lymphocytes) in lymph nodes

(Abbas et al. Cellular and Molecular Immunology, 2012)
Maturation of dendritic cells
- Ingest, kill and digest microbial pathogens
  - Primary granules
    - Cationic defensins
    - Myeloperoxidase
    - Hydrolase
    - Elastase
    - Lysozyme
  - Secondary granules
    - Iron chelators
    - Digestive enzymes
    - Lysozyme
Antigen presenting cells

**Dendritic cell**
- Antigen uptake
- Antigen presentation
- Naive T cell
- Costimulator (e.g., B7)
- CD28
- Effector T cells

**Macrophage**
- Effector T cell
- Killed microbe
- Effector T cell activation: activation of macrophages (cell-mediated immunity)

**B cell**
- Effector T cell
- Antibody
- Effector T cell activation: B cell activation and antibody production (humoral immunity)
Cells release inflammatory cytokines

1. Basophils

- Produce cytokines that help defend against parasites
- Involved in allergic inflammation
- Surface membrane receptors for IgE
- Non-phagocytic cells
- Receptor to IgE
- Released basophilic granules during type I hypersensitive reactions
2. Eosinophils

- Defend against parasites
- Involved in hypersensitivity via cytotoxicity (mediated by large granules)
- Toxic to helminths
- Receptor to IgE
- Eliminate helminths with IgE-bound
- Response to allergens

Eosinophil activation pathways

- Host protective: Defense against mucosal pathogens, cytotoxicity by granule proteins, mitochondrial DNA traps, respiratory burst, nitric oxide release
- Proinflammatory: Tissue damage and remodeling, epithelial cell damage, fibrosis, Airway hyperreactivity

Cell surface membrane
- Cytosol (most of cell contents)
- Secretory granule, with toxins
- Nucleus of cell

- T cells: Antigen presentation, Co-stimulation, Th2 polarization, recruitment
- B cells: IgM production, secretory IgA production, plasma cell survival
- Dendritic cells: Activation, maturation
3. Mast cells

- Reside in most tissues, adjacent to blood vessels
- High affinity to IgE
- Contain numerous inflammatory mediators e.g.
  - histamine
  - platelet activating factor (PAF)
  - prostaglandins
  - leukotrienes
**NK cells**

**A** NK cell activation overview

- Recognition of healthy normal cells
- Recognition of infected, damaged or tumor cells

- NK cell activation blocked
- NK cell activated
- Abnormal cell killed

**B** Inhibitory receptor engaged

- Normal cell
- Activating receptor
- Self class I MHC
- Inhibitory receptor

- NK cell not activated; no cell killing

**C** Inhibitory receptor not engaged

- Virus-infected cell
- Virus inhibits class I MHC expression

- NK cell activated; killing of infected cell

https://www.clinicalkey.com
NK cells
NKT cells (Natural killer T cells)

- Lymphocytes (0.2% of T lymphocytes)
- Contain semi-invariant receptors
  - bind lipid, glycolipid
- activate DC, NK, T and B cells
- Do not bind to MHC-I, MHC-II
- Play role in innate immunity
6. Inflammation

= body defense cell & chemicals leave the blood and enter the tissue around the injured or infected site

- Smooth muscle contract
- Endothelial cells contract, increase space btw. endothelial cells, then increased capillary permeability = vasodilation
7. Fever

TNFα, IL-1 \(\rightarrow\) the anterior hypothalamus of the brain

1. IL-1 secreted by phagocytes travels in blood to hypothalamus
2. Hypothalamus secretes prostaglandin, which resets hypothalamic thermostat
3. Nerve impulses cause shivering, higher metabolic rate, inhibition of sweating, and vasoconstriction
4. These increase body temperature to the point set by the hypothalamic thermostat

- Produce prostaglandins then increase temperature above optimum growth temp of microorganisms, effect microorganisms’ growth
- Produce heat shock proteins
- Increase enzymes activity, speed up metabolism within the body
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