Innate Immune Defenses & Protection from Parasites

Physical & chemical barriers (lysozyme, skin, mucus)
Innate cellular defenses: macrophages, neutrophils, eosinophils, basophils & mast cells
Humoral Immunity: B cells (plasma cells & memory cells)
Cell Mediated Immunity: T cells (helper & cytotoxic cells)
Host Resistance

- most pathogens (disease causing microbes)
  - must overcome surface barriers & reach underlying tissues
  - overcome resistance by host defenses

- **immune system:** nonspecific resistance & specific immune response
  - composed of widely distributed cells, tissues, & organs
  - recognizes foreign substances or microbes & acts to neutralize or destroy them

- **immunity**
  - ability of host to resist a particular disease or infection

- **immunology**
  - science concerned with immune responses

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Immunity: *Nonspecific response*

- also called nonspecific resistance, innate immunity, & natural immunity
- first line of defense
- resistance to *any* microbe or foreign material
- lacks immunological memory

**Innate Immunity**

- Physical & chemical barriers (lysozyme, skin, mucus)
- Innate cellular defenses: monocytes, macrophages, dendritic cells & granulocytes
Physical Barriers

• along with secretions (flushing) these are the first line of defense against microbes
• effectiveness impacted by:
  – direct factors
    • nutrition, physiology, fever, age, & genetics
  – indirect factors
    • personal hygiene, socioeconomic status, & living conditions

Skin

• strong mechanical barrier to microbial invasion
  – keratin produced by keratinocytes in outer layer
• inhospitable environment for microbes
  – attached organisms removed by skin shedding
  – pH is slightly acidic
  – high NaCl concentration
  – dry

Parasites that breach the skin:
• introduced by blood-sucking insect vectors (Plasmodium, Trypanosoma, Leishmania, filarial worms)
• actively invasive: hookworms, schistosomes
• exit from the skin: Guinea worm
Mucous Membranes

- protective covering that resists penetration & traps microbes
- often bathed in secretions which contain a variety of antimicrobial substances
  - Lysozyme: hydrolyzes bond connecting sugars in peptidoglycan
  - Lactoferrin: secreted by activated macrophages & PMNs, sequesters iron from plasma
  - Lactoperoxidase: produces superoxide radicals

Mucous Membranes

- line cavities exposed to the external environment
- contiguous with skin: nostrils, mouth, eyelids, genitals & anus

Parasites that breach the mucus membrane:
- orally infectious (oral-fecal cycles)
  (Giardia, Cryptosporidium, hookworms, tapeworms, Guinea worm, sheep liver fluke)
- sexually transmitted (Trichomonas)
- ocular infection (Trypanosoma cruzi)
Respiratory System

- turbulent air flow deposits microbes onto mucosal surfaces
- mucociliary blanket
  - mucous secretions that traps microbes
  - trapped microbes transported away from the lungs (mucociliary escalator)
    - expelled by coughing or sneezing
    - salivation washes microbes to stomach
- alveolar macrophages
  - phagocytic cells in alveoli of lungs

Gastrointestinal Tract

- stomach
  - gastric acid
- intestines
  - pancreatic enzymes
  - intestinal enzymes
  - bile
  - lysozyme
  - peristalsis
  - columnar epithelium shedding
  - normal microbiota
  - GALT
  - secretory IgA (this is acquired response)
Genitourinary Tract

- unfavorable environment
  - low pH of urine & vagina
  - vagina has lactobacilli
  - urea & other toxic metabolic end products in urine
  - hypertonic nature of kidney medulla
- movement of urine & mucus

Giardia is an STD
Some Schistosomes exit in the urine
Many worms produce eggs that are released in the feces

The Eye

- mucus secreting epithelial membrane
- flushing action of tears
- lysozyme, lactoferrin in tears
- secretory IgA in tears (*this is acquired response*)

- *Trypanosoma cruzi* can invade via the eye (Romana’s eye)
- *Toxoplasma gondii*, *Loa loa* and *Onchocerca* cause blindness due to damage & scarring.
Chemical Barriers

- **Gastric juices**: (stomach) pH is 1-3, pepsin, mucus
- **Peroxidase**: in saliva; produces superoxide “free” radicals that damage microbes by reacting with them.
- **Lactoferrin** found in milk and mucosal secretions (tears and saliva); sequesters iron so microbes can’t use it.
- **Mucus**: mainly composed of *mucins*, a family of large, heavily glycosylated proteins which form gels.
- **Cationic antimicrobial peptides**: highly conserved through evolution, ability to damage pathogen membranes, found in body fluids (blood, lymph)

Free radicals: are atoms, molecules or ions with unpaired electrons which cause them to be highly chemically reactive. When these react with pathogen proteins, lipids or DNA, the organism is damaged or killed.

The Complement System

- composed of >30 serum proteins
- augments (or “complements”) the antibacterial activity of antibody
- defends against pathogen infections
- bridges innate & adaptive immunity
- function as chemotactic signals to recruit phagocytes to their activation site
- puncture cell membranes causing cell lysis
- serves as opsonin to increase phagocytosis of pathogens
Complement Proteins

• Complement activation is a “cascade”
  - produced in inactive forms
  - activated following enzymatic cleavage in 
    cascade fashion
  - 3 pathways of activation
    • alternative
    • lectin
    • classical

Biochemical cascade: a series of chemical reactions in which the products of one reaction are consumed in the next reaction. These cascades generate complex molecules in small steps.

Alternative Complement Pathway

• involved in nonspecific defenses against pathogens in the circuluation
• dependent on interaction of complement with repetitive structures on pathogen surfaces
• begins with activation of C3
• results in formation of membrane attack complex
Lectin Complement Pathway

- also called the mannan-binding lectin pathway
- begins with activation of C3 & lectin binding
- dependent on interaction of host mannose-binding protein (MBP) with pathogen surfaces
  - *enhances phagocytosis*

Classical Complement Pathway

- Depends on antigen-antibody interactions: *requires acquired immunity*
- cleavage products that participate in opsonization, chemotaxis, & the membrane attack complex
- can be activated in response to microbial products

Inflammation

- Response to tissue injury
- Signs & symptoms: *redness, swelling, warmth & pain*
- Tissue responses
  - **Vasodilation** - capillary diameter increases
  - **Vascular permeability**
  - **Movement** of leukocytes (immune cells) into tissues
Inflammatory Mediators

- Soluble, diffusible molecules that act at the site of tissue damage or infection, and at more distant sites.
- Histamine: vasodilation & capillary permeability
- Arachidonic acid converted to prostaglandins $\rightarrow$ tissue swelling & pain

Function of Inflammation

Increased blood flow $\rightarrow$ Increased numbers of immune system cells
Increased temperature $\rightarrow$ Inhibits microbial growth
Chemotactic factors $\rightarrow$ Attract phagocytes to trap & kill microbes (by phagocytosis).

Chemotaxis by WBC  Macrophage phagocytosis
Phagocytosis

- Monocytes, macrophages & neutrophils phagocytose
- Requires attachment to microbe
- Microbe contained in membrane vesicle (phagosome)
- Microbe digested by lysosomal enzymes

1. Attachment

2. Phagosome formation

3. Phagosome-lysosome fusion

4. Digestion

Opsonization - Enhanced Attachment
Innate cellular defenses: monocytes, macrophages, dendritic cells & granulocytes (neutrophils, eosinophils, basophils & mast cells)

Leukocytes: White Blood Cells

Mononuclear cells:
Monocyte-Macrophage system

Granulocytic cells:
(PMNs = polymorphonuclear leukocytes)
Neutrophils, Eosinophils, Basophils & Mast cells

Lymphoid cells:
T & B lymphocytes
Cells of the Immune System

- Innate Immune Response
  - *Immediate response*: recognizes distinctive properties of pathogens
  - Effector cells
    - Granulocytes
    - Mast cells
    - Monocytes, macrophages & dendritic cells

- Acquired Immune Response
  - *Delayed response*: recognizes specific properties of individual pathogens
  - Has memory
  - Effector cells
    - B lymphocytes
    - T lymphocytes
    - Interacts with innate immune cells

Phagocytosis

A process by which phagocytic cells (monocytes, macrophages, dendritic cells, & neutrophils) recognize, ingest, & kill extracellular microbes
Phagocytosis

- two mechanisms for recognition of microbe by phagocyte
  - opsonin-independent (nonopsonic) recognition
    - pathogen recognition
    - common pathogen components (i.e. terminal sugars) are non-specifically recognized to activate phagocytes
    - signaling mechanism involved
  - opsonin-dependent (opsonic) recognition

Neutrophils

- highly phagocytic & highly motile (chemotactic)
- circulate in blood, migrate to sites of tissue damage
- kill ingested microbes with lytic enzymes & reactive oxygen metabolites contained in primary & secondary granules
- a pathogen is likely to first encounter a neutrophil.

- Neutrophil chemotaxis: migration toward sites of infection or inflammation.
- Neutrophils detect gradients of cytokines which are used to direct the path of their migration
- Neutrophils can be a host cell for Leishmania (protozoan parasite)
- Neutrophils detect Wolbachia bacteria associated with helminths & while trying to eliminate it, as a consequence, they conceal the helminth from the immune system!
Basophils

- nonphagocytic
- release vasoactive mediators
  - e.g., histamine, prostaglandins, serotonin, & leukotrienes from granules
- role in development of allergies & hypersensitivities

Histamine increases capillary permeability to white blood cells and proteins, to allow them to engage pathogens in the infected tissues. Prostaglandins promote inflammation. Leukotrienes increase permeability of small blood vessels, enhance secretion of mucus in the airway & gut & recruit leukocytes to sites of inflammation. These are especially important to a response to worms.

Eosinophils

- defend against protozoan & helminth parasites
- release cationic proteins & reactive oxygen metabolites (ROIs)
- may play a role in allergic reactions

- Reactive oxygen species (ROS): chemically reactive molecules containing oxygen (oxygen ions & peroxides). ROS are highly reactive due to the presence of unpaired valence shell electrons resulting in damage to cells.
- Very important to combat helminthic infections: release toxic components when IgE antibody binds to helminth
- tissue-invasive helminthic parasites cause eosinophilia (increased eosinophils)
Mast Cells

- contain granules containing histamine & other pharmacologically active chemicals
- Important in protection from helminth infection
- play important role in development of allergies & hypersensitivities
- similar in appearance & function to basophils,
- circulates in immature form, matures once in tissue

- *Mast cells are present in most tissues & are especially prominent near the boundaries to the outside world (skin, lung mucosa, digestive tract, mouth, conjunctiva & nose."
- Degranulation releases toxic components
- Very important to combat helminth infections: release toxic components when IgE antibody binds to helminth

ADCC - Antibody-depdendant cellular cytotoxicity
Monocytes & Macrophages

- highly phagocytic cells
- Monocytes mature into macrophages after circulating for ~8 hours
- Macrophages: 5-10X larger than monocytes, reside in specific tissues, highly phagocytic
  - named according to tissue in which they reside
  - surface receptors recognize pathogen associated molecular patterns (PAMPs)

In addition to controlling infection by protozoa, macrophages are host cells for several protozoan parasites (Leishmania, Toxoplasma, T. cruzi). This permits the parasites to disseminate in the body.

Dendritic Cells (DCs)

- heterogeneous group with neuron-like appendages
- present in small numbers in blood, skin, & mucous membranes of nose, lungs, & intestines
  - express pattern recognition receptors
  - contact, phagocytose, & process antigens → display foreign antigens on their surfaces (antigen presentation)

- DCs are critical to initiate cellular immunity against parasites & in directing Th1/Th2 choice
- Intracellular parasites such as Leishmania can down-regulate the ability of DCs to present antigen
Pathogen-Associated Molecular Patterns (PAMPs)

- Phagocytes detect conserved microbial molecular structures that occur in patterns
- PAMPs are unique to microbes, not present in host
  - e.g., GPI anchors in protozoan parasites
  - e.g., carbohydrates that are distinct from vertebrate forms
- PAMPs recognized by pattern recognition receptors (PRRs) on phagocytic cells
- **Toll-Like Receptors** (TLRs) are a class of PRRs that recognize & bind PAMPs
- binding triggers an evolutionarily ancient signal & is communicated to the host cell nucleus which initiates the host response

Intracellular Digestion

- microbes are internalized into a **phagosome**
  - **respiratory burst reactions** occur as soon as phagosome is formed
  - **toxic oxygen products** are produced which can kill invading microbes
- Phagolysosome: fusion of phagosome with lysosome
  - presence of toxic chemicals
  - e.g., digestive enzymes
  - e.g., toxic reactive oxygen intermediates (ROIs)
  - e.g., reactive nitrogen intermediates (RNIs)
Parasites & Macrophages

- Many pathogens hide out inside of neutrophil or macrophages: a Trojan horse strategy
- Some do not enter through phagocytosis (*Toxoplasma*)
- Others modify the outcome of phagocytosis
  - resistant to phagosomal killing (*Leishmania*)
  - escape to cytoplasm (*Trypanosoma cruzi*)

Toxoplasma & Macrophages

- active invasion is more rapid than phagocytosis → it’s a race between the parasite and the host cell
- respiratory burst not triggered after invasion
- parasite grows in a membrane bound vacuole
  - resists fusion with endosomes & lysosomes
  - associates with host cell ER and mitochondria.

- [video 1](http://www.youtube.com/watch?v=5qHNoTZMz6w&feature=BFa&list=UL6-Hh2YFl2FI)
- [video 2](http://www.youtube.com/watch?v=1x1220lqkx4&feature=channel&list=UL)
**Leishmania & Macrophages**

- *Leishmania* make lipopolyglycan (LPG), a complex molecule with both lipid & sugar components
- LPG modulates the host immune response
  1. resists complement-mediated lysis (MAC)
  2. inhibits the oxidative burst
  3. prevents natural killer T cells from recognizing that the macrophage is infected

**Trypanosoma cruzi & Macrophages**

- *T. cruzi* mainly grows in cardiac myocytes or macrophages
- can enter host cells either by active invasion or by phagocytosis (depends on host cell type)
  - degrades lysosomal proteases
  - escapes from acidified, membrane-bound vacuole into the cytoplasm where it replicates
- [http://www.youtube.com/watch?v=7q9PTljtRhE](http://www.youtube.com/watch?v=7q9PTljtRhE)