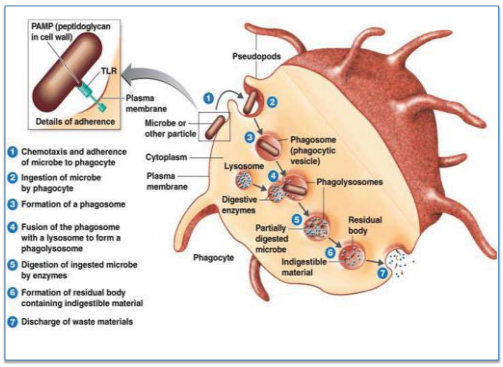
Mechanisms of innate immunity

1. Phagocytosis

- **Phagocytosis is the cellular uptake (eating) of particulate materials such as bacteria—a key mechanism for eliminating pathogens.**
- **Derived from the Greek words "Eat and cell".**
- **Phagocytosis is carried out by white blood cells:**
- **Macrophages, neutrophils (microphage), and dendritic cells in tissues and monocytes in the blood are the main cell types that carry out phagocytosis and occasionally eosinophils .**
- **Neutrophils predominate early in infection.**
- **Wandering macrophages: Originate from monocytes that leave blood and enter infected tissue, and develop into phagocytic cells.**
- **Fixed Macrophages (Histiocytes): Located in liver, nervous system, lungs, lymph nodes, bone marrow, and several other tissues.**
- **Phagocytic cells make up the next line of defense against pathogens that have penetrated the epithelial cell barriers.**
- **Elie Metchnikoff initially described the process of phagocytosis in the 1880s .**

SECOND LECTURE



Stages of Phagocytosis

1. Chemotaxis: Phagocytes are chemically attracted to site of infection
– Cytokines (released from other WBCs)
– Cell damage
– Microbial products
2• Adherence: Phagocyte plasma membrane attaches to surface of pathogen or foreign material.
– Toll-like receptors (TLRs)
– Pathogen associated Molecular Patterns (PAMPs)
Opsonins proteins that coat microbe which include antibodies and complement proteins

Opsonization: Coating process with opsonins that facilitates attachment.

3. Ingestion: Plasma membrane of phagocytes extends projections (pseudopods) which engulf the microbe. Microbe is enclosed in a sac called phagosome.

4. Digestion: Inside the cell, phagosome fuses with lysosome to form a phagolysosome.

Lysosomal enzymes kill most bacteria within 30 minutes and include:

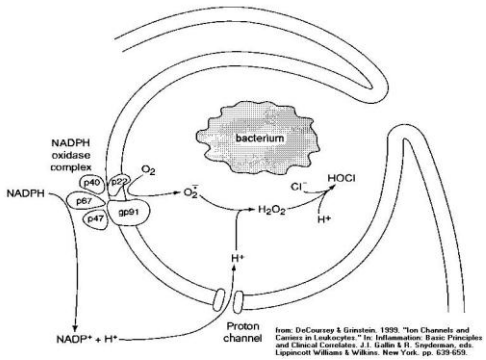
Lysozyme: Destroys cell wall peptidoglycan

Lipases and Proteases

RNAses and DNAses

After digestion, residual body with undigestable material is discharg

Respiratory burst



Mechanisms of killing during phagocytosis
Oxygen-dependent intracellular

- When a phagocyte ingests bacteria (or any material) , its oxygen consumption increases. The increase in oxygen consumption, called a respiratory burst, produces reactive oxygen-containing molecules that are anti-microbial.
- The oxygen compounds are toxic to both the invader and the cell itself, so they are kept in compartments inside the cell. This method of killing invading microbes by using the reactive oxygen-containing molecules is referred to as oxygen-dependent intracellular killing, of which there are two types.

- The first type is the oxygen-dependent production of a superoxide, which is an oxygen-rich bacteria-killing substance. The superoxide is converted to hydrogen peroxide and singlet oxygen by an enzyme called superoxide dismutase. Superoxides also react with the hydrogen peroxide to produce hydroxyl radicals, which assist in killing the invading microbe.
- The second type involves the use of the enzyme myeloperoxidase from neutrophil granules. When granules fuse with a phagosome, myeloperoxidase is released into the phagolysosome, and this enzyme uses hydrogen peroxide and chlorine to create hypochlorite, a substance used in domestic bleach. Hypochlorite is extremely toxic to bacteria .
- Myeloperoxidase contains a heme pigment, which accounts for the green color of secretions rich in neutrophils, such as pus and infected sputum.

Killing by reactive nitrogen intermediates (RNI)

- The generation of RNS requires the transcriptional activation of the gene for the enzyme **inducible nitric oxide synthase (iNOS,** or **NOS2)**—called that to distinguish it from related NO synthases in other tissues.
- Expression of iNOS is activated by microbial components binding to various PRRs. iNOS oxidizes L-arginine to yield L-citrulline and nitric oxide (NO), a potent antimicrobial agent.
- Whereas the NADPH oxidase is dedicated to the killing of extracellular organisms taken up by phagocytosis and cornered within the phagocytic vacuole, the NO · mechanism can operate against microbes that invade the cytosol; so, it is not surprising that the majority of non phagocytic cells that may be infected by viruses and other parasites are endowed with an inducible NO · synthase (iNOS) capability.
- The mechanism of action may be through degradation of the Fe – S prosthetic groups of certain electron transport enzymes, depletion of iron and production of toxic· ONOO radicals.

Oxygen independent killing or Killing by preformed antimicrobials

- Phagocytes can also kill microbes by oxygen-independent methods, but these are not as effective as the oxygen-dependent ones.
- These molecules, contained within the neutrophil granules, contact the ingested microorganism when fusion with the phagosome occurs.

- The pH of the phagolysosome may be as low as 4.0 due to the dismutation of superoxide consumes hydrogen ions accumulation of lactic acid, which is sufficiently acidic to prevent the growth of most pathogens. This acidic environment apparently optimizes the activity of many degradative lysosomal enzymes including lysozyme, glycosylases, phospholipases, and nucleases
- There are four main types.
- The first uses electrically charged proteins that damage the bacterium's membrane.
- The second type uses lysozymes; these enzymes break down the bacterial cell wall.
- The third type uses lactoferrins, which are present in neutrophil granules and remove essential iron from bacteria.
- The fourth type uses proteases and hydrolytic enzymes; these enzymes are used to digest the proteins of destroyed bacteria.

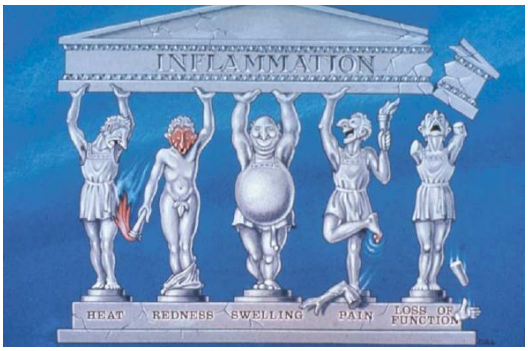
Inflammatory Responses (inflammation)

When the outer barriers of innate immunity—skin and other epithelial layers—are damaged, the resulting innate responses to infection or tissue injury can induce a complex cascade of events known as the **inflammatory response**. Inflammation may be acute (short-term effects contributing to combating infection, followed by healing)—for example, in response to local tissue damage—or it may be chronic (long term, not resolved), contributing to conditions such as arthritis, inflammatory bowel disease, cardiovascular disease, and Type 2 diabetes.

Opsonization

- Activation of phagocytosis can also occur indirectly, by phagocyte recognition of soluble proteins that have bound to microbial surfaces, thus enhancing phagocytosis, a process called **opsonization** (from the Greek word for "to make tasty").
- Many of these soluble phagocytosis-enhancing proteins (called **opsonins**) also bind to components on the surfaces of microbes such as
- a-carbohydrate structures,
- b-lipopolysaccharides, and
- c-viral proteins.
- Once bound to microbe surfaces, opsonins are recognized by membrane opsonin receptors on phagocytes, activating phagocytosis

Hallmarks of inflammation



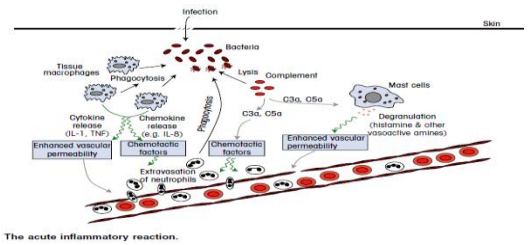
The hallmarks of a localized inflammatory response were first described by the Roman physician Celsus in the first century AD as *rubor et tumor cum calore et dolore* (redness and swelling with heat and pain). An additional mark of infl ammation added in the second century by the physician Galen is loss of function (*functio laesa*). Today we know that these symptoms reflect an increase in vascular diameter (vasodilation), resulting in a rise of blood volume in the area.

- Recruited leukocytes are activated to phagocytose bacteria and debris and to amplify the response by producing additional mediators.
- Resolution of this acute inflammatory response includes
- the clearance of invading pathogens,
- dead cells, and damaged tissue;
- the activation of the systemic acute phase response and additional physiological responses, including the initiation of wound healing; and the induction of adaptive immune responses.

Higher blood volume heats the tissue and causes it to redden. Vascular permeability also increases, leading to leakage of fluid from the blood vessels, resulting in an accumulation of fluid (**edema**) that swells the tissue. Within a few hours, leukocytes also enter the tissue from the local blood vessels. These hallmark features of inflammatory responses reflect the activation of resident tissue cells—macrophages, mast cells, and dendritic cells—by PAMPs and DAMPs to release chemokines, cytokines, and other soluble mediators into the vicinity of the infection or wound.

- **The recruitment of various leukocyte populations to the site of infection or damage is a critical early component of inflammatory responses.**
- **PRR signaling activates resident macrophages, dendritic cells, and mast cells to release the initial components of cellular innate immune responses, including the**
- **proinflammatory cytokines TNF-α , IL-1, and IL-6;**
- **chemokines;**
- **prostaglandins ; and**
- **histamine and other mediators released by mast cells.**
- **These factors act on the vascular endothelial cells of local blood vessels, increasing vascular permeability and the expression of cell adhesion molecules (CAMs) and chemokines such as IL-8.**

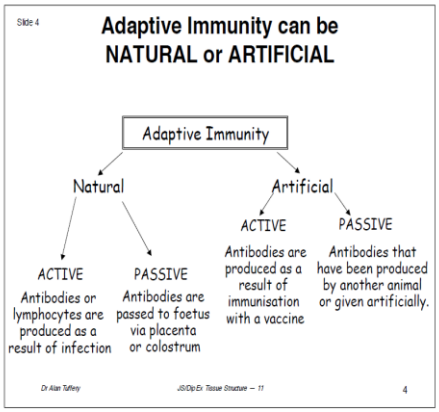
However, if the infection or tissue damage is not resolved, it can lead to a chronic inflammatory state that can cause more local tissue damage and potentially have systemic consequence .



The acute inflammatory reaction.

Neutrophils are the first to be recruited to a site of infection where they enhance local innate responses, followed by monocytes that differentiate into macrophages that participate in pathogen clearance and help initiate wound healing.

In addition to these key events at the site of infection or damage, the key cytokines made early in response to innate and inflammatory stimuli—TNF-α, IL-1, and IL-6—also have systemic effects.
They induce fever (a protective response, as elevated body temperature inhibits replication of some pathogens) by inducing COX-2 expression, which activates prostaglandin synthesis, as mentioned above. Prostaglandin E2 (PGE2) acts on the hypothalamus (the brain center controlling body temperature), causing fever. These three proinflammatory cytokines also act on the liver, inducing the acute phase response, which contributes to the resolution of the inflammatory response.



Features of Adaptive Immunity

- Specificity
  - Lymphocytes (B and T cells) bind and respond to foreign molecules (antigens) via antigen receptors: each to a specific antigen
- Diversity
  - The body possesses millions of lymphocytes that can recognize and respond to millions of antigens (one each)
- Memory
  - 1st exposure to an antigen generates lymphocytes & long-lived memory cells – next exposure to the same antigen, memory cells react more quickly & stronger response ('acquired immunity')
- Self-Tolerance
  - Lymphocytes can distinguish 'self' (our normal antigens) from 'nonself' (antigens from foreign material).

TABLE 5-1 Innate and adaptive immunity

Attribute	Innate immunity	Adaptive immunity
Response time	Minutes/hours	Days
Specificity	Specific for molecules and molecular patterns associated with pathogens and molecules produced by dead/damaged cells	Highly specific; discriminates between ev minor differences in molecular structure o microbial or nonmicrobial molecules
Diversity	A limited number of conserved, germ line–encoded receptors	Highly diverse; a very large number of receptors arising from genetic recombinat of receptor genes in each individual
Memory responses	Some (observed in invertebrate innate responses and mouse/human NK cells)	Persistent memory, with faster response o greater magnitude on subsequent exposu
Self/nonself discrimination	Perfect; no microbe-specific self/nonself patterns in host	Very good; occasional failures of discrimination result in autoimmune dise
Soluble components of blood	Many antimicrobial peptides, proteins, and other mediators	Antibodies and cytokines
Major cell types	Phagocytes (monocytes, macrophages, neutrophils), natural killer (NK) cells, other leukocytes, epithelial and endothelial cells	T cells, B cells, antigen-presenting cells

Table 1-3 Comparison of Types of Acquired Immunity

	Type	Mode of Acquisition	Antibody Produced by Host	Duration of Immune Response
Active	Natural	Infection	Yes	Long*,†
	Artificial	Vaccination	Yes	Long*,†
Passive	Natural	Transfer in vivo or colostrum	No	Short
	Artificial	Infusion of serum/plasma	No	Short

*Immunocompetent host.
†IgG immune antibody half-life is 23 days. Memory cells (memory lymphocytes) lifespan is years.