

Unit 5:

Innate Immunity

I. An Overview of Innate and Adaptive Immunity
Innate Immunity

II. Innate Immunity

An Overview of Innate and Adaptive Immunity



1. The body has two immune systems: the innate immune system and the adaptive immune system.
2. Innate immunity is an antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to almost any microbe.
3. Innate immunity is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.
4. Immediate innate immunity begins 0 - 4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood and in extracellular tissue fluids.
5. Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPs binding to pattern-recognition receptors or PRRs.
6. Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to react with and remove a specific antigen.
7. Adaptive immunity is the immunity one develops throughout life.
8. An antigen is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes.
9. The actual portions or fragments of an antigen that react with antibodies and lymphocyte receptors are called epitopes.

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1. Cite the differences between innate and adaptive (acquired) immunity
2. Identify places where innate and adaptive immunity intersect.

Detailed Learning Objectives

- 1**. Compare adaptive (acquired) immunity with innate immunity.
- 2*. Compare immediate innate immunity with early induced innate immunity.
3. Define the following:
 - a*. pathogen-associated molecular patterns (PAMPs)
 - b*. pattern-recognition receptors (PRRs)
 - c*. antigen
 - d*. immunogen
 - e*. epitope.

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Questions

An Overview of Innate and Adaptive Immunity

The body has two immune systems: the **innate immune system** and the **adaptive immune system**. Unit 5 deals with innate immunity while Unit 6 will cover adaptive immunity. Let's first briefly compare acquired and innate immunity.

1. Innate Immunity

Innate immunity is an antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to almost any microbe. This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection. Innate immunity can be divided into immediate innate immunity and early induced innate immunity.

a. Immediate innate immunity

Immediate innate immunity **begins 0 - 4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood, our found in extracellular tissue fluids, and are secreted by epithelial cells.** These include:

- antimicrobial enzymes and peptides;
- complement system proteins; and
- anatomical barriers to infection, mechanical removal of microbes, and bacterial antagonism by normal body microbiota.

These preformed innate defense molecules will be discussed in greater detail later in this unit.

b. Early induced innate immunity

Early induced innate immunity **begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPS binding to pattern-recognition receptors or PRRs.** These recruited defense cells include:

- phagocytic cells: leukocytes such as neutrophils, eosinophils, and monocytes; tissue phagocytic cells in the tissue such as macrophages;
- cells that release inflammatory mediators: inflammatory cells in the tissue such as macrophages and mast cells; leukocytes such as basophils and eosinophils; and
- natural killer cells (NK cells).

Unlike adaptive immunity, innate immunity does not recognize every possible antigen. Instead, it is **designed to recognize molecules shared by groups of related microbes that are essential for the survival of those organisms** and are not found associated with mammalian cells. These unique microbial molecules are called **pathogen-associated molecular patterns** or PAMPS and include LPS from the gram-negative cell wall, peptidoglycan and lipotechoic acids from the gram-positive cell wall, the sugar mannose (a terminal sugar common in microbial glycolipids and glycoproteins but rare in those of humans), bacterial and viral unmethylated CpG DNA, bacterial flagellin, the amino acid *N*-formylmethionine found in bacterial proteins, double-stranded and single-stranded RNA from viruses, and glucans from fungal cell walls. In addition, unique molecules displayed on stressed, injured, infected, or transformed human cells also act as PAMPS. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometimes referred to as microbe-associated molecular patterns or MAMPs.)

Most body defense cells have pattern-recognition receptors or PRRs for these common PAMPS (**see Fig. 1**) and so there is an **immediate response** against the

and remove a specific antigen. This is the immunity one develops throughout life.

During adaptive immunity, antigens are transported to lymphoid organs where they are recognized by naive B-lymphocytes and T-lymphocytes. These activated B- and T-lymphocytes subsequently proliferate and differentiate into effector cells.

An **antigen** is defined as **a substance that reacts with antibody molecules and antigen receptors on lymphocytes**. An **immunogen** is **an antigen that is recognized by the body as nonself and stimulates an adaptive immune response**. For simplicity we will use the term antigen when referring to both antigens and immunogens. The actual portions or fragments of an antigen that react with antibodies and lymphocyte receptors are called **epitopes**.

For more information: Preview of antigens, epitopes, and immunogens
For more information: Preview of antibodies

The body recognizes an antigen as foreign when **epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope**. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

It is estimated that the human body has the ability to recognize 10^7 or more different epitopes and make up to 10^9 different antibodies, each with a unique specificity. In order to recognize this immense number of different epitopes, the body produces 10^7 or more distinct clones of both B-lymphocytes and T-lymphocytes, each with a unique B-cell receptor or T-cell receptor. Among this large variety of B-cell receptors and T-cell receptors there is bound to be at least one that has an epitope-binding site able to fit, at least to some degree, any antigen the immune system eventually encounters. With the adaptive immune responses, the body is able to **recognize any conceivable antigen it may eventually encounter**.

The downside to the specificity of adaptive immunity is that only a few B-cells and T-cells in the body recognize any one epitope. **These few cells then must rapidly proliferate in order to produce enough cells to mount an effective immune response** against that particular epitope, and that **typically takes several days**. During this time the pathogen could be causing considerable harm, and that is why innate immunity is also essential.

For more information: Preview of B-Lymphocytes
For more information: Preview of T4-Lymphocytes
For more information: Preview T8-Lymphocytes

Flash animation showing epitopes reacting with specific B-cell receptor on a B-lymphocytes
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing epitopes reacting with specific B-cell receptor on a B-lymphocytes
B-lymphocytes have B-cell receptors (BCRs) corresponding to the specific antibody molecule they are genetically programmed to make. These BCRs recognize epitopes of antigens having a complementary shape. Different B-lymphocytes are programmed to produce different BCRs, each specific for a unique epitope.

Flash animation showing epitopes reacting with a specific TCR on a T8-lymphocyte
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing epitopes reacting with a specific TCR on a T8-lymphocyte
Naive T8-lymphocytes via the unique T-cell receptors and CD8 molecules on their surface recognize peptide epitopes from endogenous antigens bound to MHC-I molecules on antigen presenting cells (APCs). Different T-cell receptors recognize different epitopes.

Adaptive immunity usually improves upon repeated exposure to a given infection and involves the following:

- antigen-presenting cells (APCs) such as macrophages and dendritic cells;
- the activation and proliferation of antigen-specific B-lymphocytes;
- the activation and proliferation of antigen-specific T-lymphocytes; and
- the production of antibody molecules, cytotoxic T-lymphocytes (CTLs), activated macrophages, and cytokines.

There are **two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity**.

1. **Humoral immunity:** Humoral immunity involves the production of **antibody molecules in response to an antigen** and is mediated by B-lymphocytes.

Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes. For example, antibodies made against cell wall antigens can stick bacteria to phagocytes, a process called opsonization; antibodies made against cell wall adhesins can prevent bacteria from adhering to and colonizing host cells; antibodies against viral surface antigens can block viral adsorption to host cells; antibodies against exotoxins can neutralize these toxins.

2. **Cell-mediated immunity:** Cell-mediated immunity involves **the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen** and is mediated by T-lymphocytes. These defense cells help to **remove infected cells and cancer cells displaying foreign epitopes**.

Adaptive immunity will be discussed in detail in Unit 6.

Concept Map for Innate Versus Adaptive Immunity
TPS Questions

Self Quiz for an Overview of Innate and Adaptive Immunity

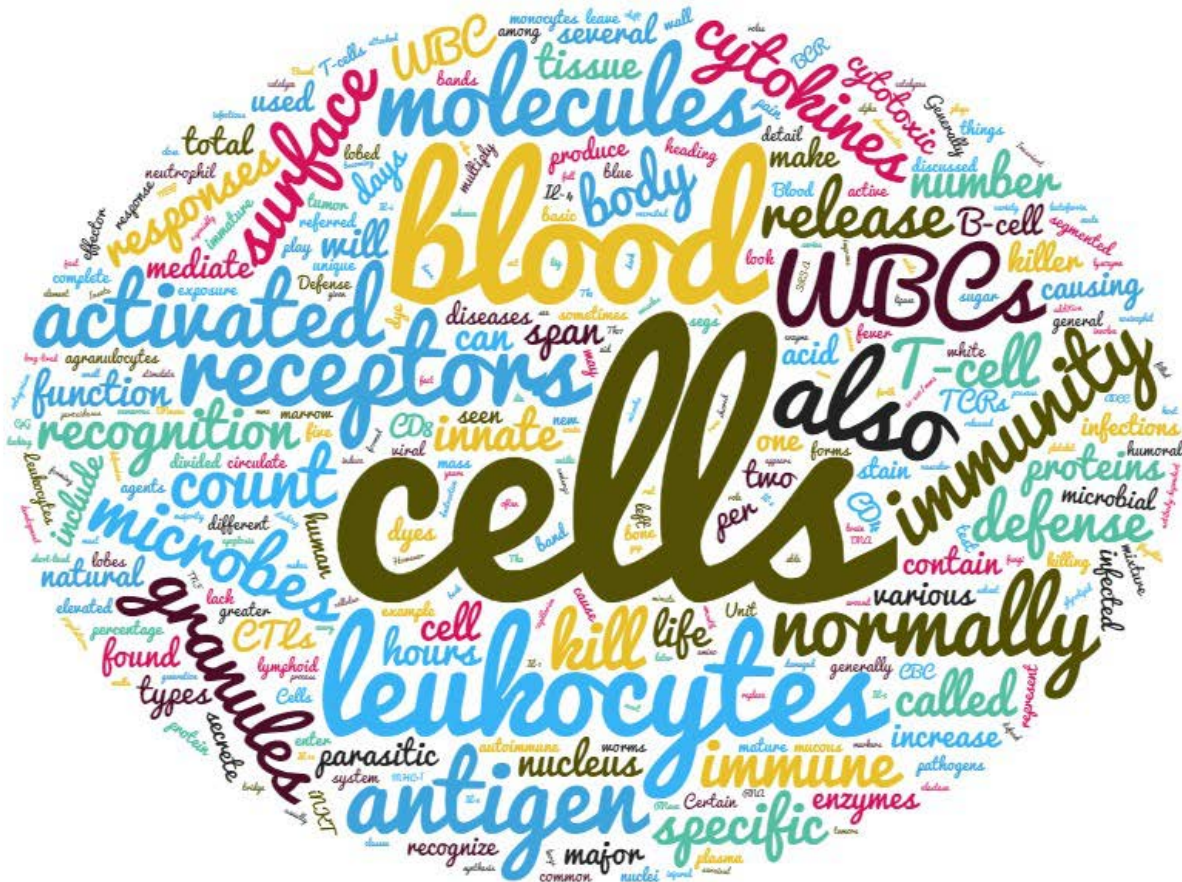


[Return to Unit 5 and 6 Table of Contents](#)

[Back to Softchalk Lessons Table of Contents](#)

Innate Immunity

Defense Cells in the Blood: The Leukocytes



1. The complete blood count (CBC) is a laboratory test that, among other things, determines the total number of both leukocytes and erythrocytes per ml of blood.
2. In general, an elevated WBC count (leukocytosis) is seen in infection, inflammation, leukemia, and parasitic infestations.
3. Neutrophils are the most abundant of the leukocytes, normally accounting for 54-75% of the WBCs. Neutrophils are important phagocytes and also promote inflammation.
4. Eosinophils normally comprise 1-4% of the WBCs. They are capable of phagocytosis but primarily they release their contents into the surrounding environment to kill microbes, especially parasitic worms, extracellularly. They also promote inflammation.
5. Basophils normally make up 0-1% of the WBCs and release histamine, leukotrienes, and prostaglandins, chemicals that promote inflammation.
6. Monocytes normally make up 2-8% of the WBCs and differentiate into macrophages and dendritic cells when they leave the blood and enter the tissue.
7. Lymphocytes normally represent 25-40% of the WBCs and mediate the specific immune responses.
8. B-lymphocytes (B-cells) mediate humoral immunity, the production of antibody molecules against a specific antigen, and have B-cell receptors (BCR) on their surface for antigen recognition. Most B-lymphocytes differentiate into antibody-secreting plasma cells.
9. T-lymphocytes (T-cells) are responsible for cell-mediated immunity, the production of cytotoxic T-lymphocytes (CTLs), activated macrophages, activated NK cells, and cytokines against a specific antigen.
10. T4-lymphocytes have CD4 molecules and T-cell receptors on their surface for antigen recognition. They function to regulate the adaptive immune responses through cytokine production. Once activated, they differentiate into effector T4-lymphocytes.
11. T8-lymphocytes have CD8 molecules and T-cell receptors on their surface for antigen recognition. Once activated, they differentiate into T8-suppressor cells and cytotoxic T-lymphocytes (CTLs).

12. *NK cells (natural killer cells) are lymphocytes that lack B-cell receptors and T-cell receptors. They function to kill infected cells and tumor cells.*

Common Course Objectives

- 1. Explain the role each of the different types of blood cells play in immunity

Detailed Learning Objectives

- 1. State what each of the following determine: CBC and leukocyte differential count.
- 2. State the significance of the following:
 - a*. an elevated white blood cell count
 - b*. a shift to the left (elevated bands)
- 3**.Describe and state the major functions of the following leukocytes:

- a. neutrophils
- b. basophils
- c. eosinophils
- d. monocytes
- e. B-lymphocytes
- f. T4-lymphocytes
- g. T8-lymphocytes
- h. NK cells

- 4. State what type of cell monocytes differentiate into when they enter tissue.
- 5. State 2 functions of platelets.

(*) = Common theme throughout the course
(**) = More depth and common theme

TPS Questions

Defense Cells in the Blood: The Leukocytes

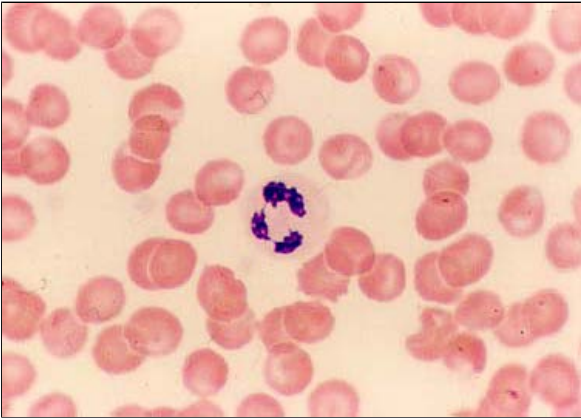
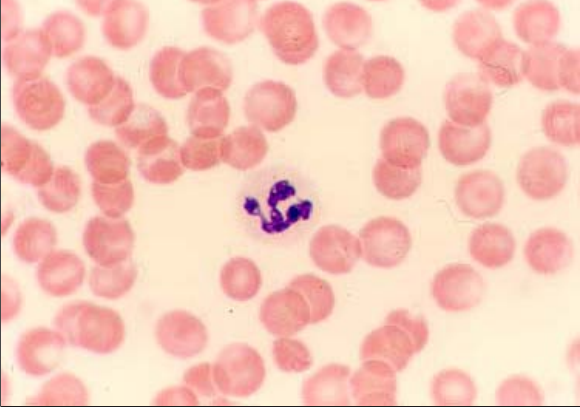
All leukocytes are critical to body defense. There are normally between 5,000-10,000 leukocytes per cubic millimeter (mm³) of blood and these can be divided into five major types: neutrophils, basophils, eosinophils, monocytes, and lymphocytes. The production of colonies of the different types of leukocytes is called leukopoiesis and is induced by various cytokines known as **colony stimulating factors** or **CSFs**.

3D animation illustrating colony-stimulating factors

From Drew Berry, wehi.edu.au. This animation takes some time to load.

The **complete blood count (CBC)** is a laboratory test which, among other things, determines the **total number of both leukocytes and erythrocytes** per ml of blood. In general, an elevated WBC count (leukocytosis) is seen in infection, inflammation, leukemia, and parasitic infestations. A decreased WBC count (leukopenia) is generally seen in bone marrow depression, severe infection, viral infections, autoimmune diseases, malignancies, and malnutrition. For example, infections may increase the total leukocyte count two to three times the normal level by dramatically increasing the number of neutrophils.

The **differential white blood cell count (leukocyte differential count)** determines the **number of each type of leukocyte** calculated as a percentage of the total number of leukocytes. This information can be useful diagnostically because different diseases or disorders can cause an increase or a decrease in the various types of WBCs. For example, when doing a differential WBC count, neutrophils are usually divided into **segs** (a mature neutrophil having a segmented nucleus; **see Fig. 1A**) and **bands** (an immature neutrophil with an incompletely segmented or banded nucleus; **see Fig. 1B**). During an **active infection**, people are generally producing **large numbers of new neutrophils** and therefore will have a **higher percentage of the immature band forms**. (An increase in band forms is sometimes referred to as a "**shift to the left**" because on laboratory slips used for differential WBC counts, the heading for bands is to the left of the heading for mature neutrophils or segs.)

Fig. 1: Photomicrograph of a Mature or Segmented Neutrophil	Fig. 1B: Photomicrograph of an Immature or Band Form Neutrophil
	
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The nucleus appears multilobed and segmented with only thin threads connecting the lobes.	The nucleus appears multilobed and segmented with thick bands connecting the lobes.



The five types of leukocytes fall into one of two groups, the polymorphonuclear leukocytes and the mononuclear leukocytes.

a. Polymorphonuclear leukocytes (granulocytes)

Polymorphonuclear leukocytes (granulocytes) have **irregular shaped nuclei with several lobes** and their **cytoplasm is filled with granules** containing enzymes and antimicrobial chemicals. They include the following:

1. neutrophils

Neutrophils are the most abundant of the leukocytes, normally accounting for 54-75% of the WBCs. An adult typically has 3,000-7,500 neutrophils/mm³ of blood but the number may increase two- to three-fold during active infections. They are called neutrophils because their granules stain poorly - they have a neutral color - with the mixture of dyes used in staining leukocytes. The nucleus of a neutrophil has multiple lobes (**see Fig. 1A and Fig. 1B**).

Fig. 1: Photomicrograph of a Mature or Segmented Neutrophil	Fig. 1B: Photomicrograph of an Immature or Band Form Neutrophil
	
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The nucleus appears multilobed and segmented with only thin threads connecting the lobes.	The nucleus appears multilobed and segmented with thick bands connecting the lobes.

Functions of neutrophils:

- a. Neutrophils are important **phagocytes**.
- b. Their **granules contain various agents for killing microbes**. Primary azurophil granules contain acid hydrolase, myeloperoxidase, defensins, cathepsin

G, cationic proteins, and bactericidal permeability increasing protein (BPI). Secondary specific granules contain such defense chemicals as lysozyme, lactoferrin, collagenase, and elastase. These agents kill microbes intracellularly during phagocytosis but are also often released extracellularly where they kill not only microbes but also surrounding cells and tissue, as will be discussed later under phagocytosis.

c. They **release the enzyme kallikrein** that catalyzes the generation of **bradykinins**. Bradykinins **promote inflammation** by causing vasodilation, increasing vascular permeability, and increasing mucous production. They are also chemotactic for leukocytes and stimulate pain.

d. They **produce enzymes** that catalyze the synthesis of **prostaglandins** from arachidonic acid in cell membranes. Certain prostaglandins **promote inflammation** by causing vasodilation and increasing capillary permeability. They also cause constriction of smooth muscles, enhance pain, and induce fever.

e. They are short-lived, having a life span of a few hours to a few days, and do not multiply. They circulate in the blood for around 6 hours and if they are not recruited, they undergo apoptosis. In tissue, they function for several hours and die. However, the bone marrow makes about 80,000,000 new neutrophils per minute to replace these.

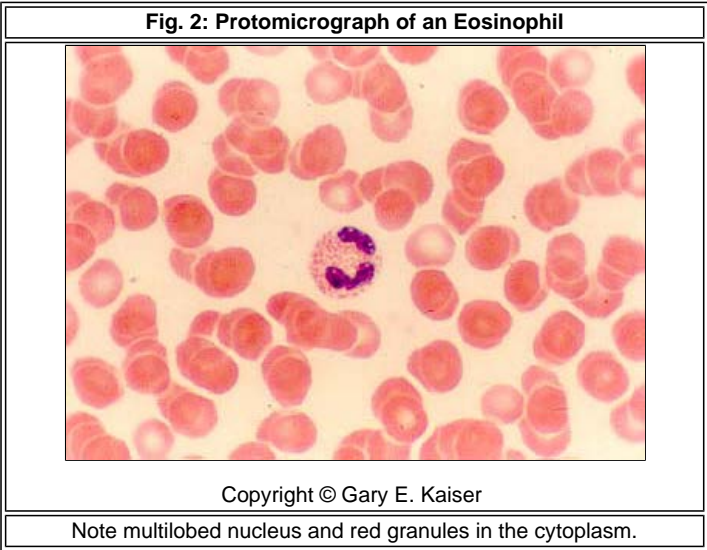
Electron micrograph of a neutrophil, from the University of Illinois College of Medicine.

Scanning electron micrograph of a neutrophil engulfing *Escherichia coli* from sciencephotogallery.com.

Transmission electron micrograph of a neutrophil engulfing *Neisseria gonorrhoeae* from sciencephotogallery.com.

2. eosinophils

Eosinophils normally comprise 1-4% of the WBCs (50-400/mm³ of blood). They are called eosinophils because their granules stain red with the acidic dye eosin, one of the mixture of dyes used when staining leukocytes. The nucleus of an eosinophil typically appears lobed (see Fig. 2).



a. Their **granules contain destructive enzymes for killing infectious organisms**. These enzymes include acid phosphatase, peroxidases, major basic protein, RNase, DNases, lipase, and plasminogen.

b. They are capable of **phagocytosis** but primarily they release their contents into the surrounding environment to kill microbes extracellularly.

c. The substances they release defend primarily against fungi, protozoa, and parasitic worms (helminths), pathogens that are too big to be consumed by phagocytosis.

d. They secrete **leukotrienes, prostaglandins**, chemicals that **promotes inflammation** by causing vasodilation and increasing capillary permeability. They also secrete various cytokines such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-13, and TNF alpha.

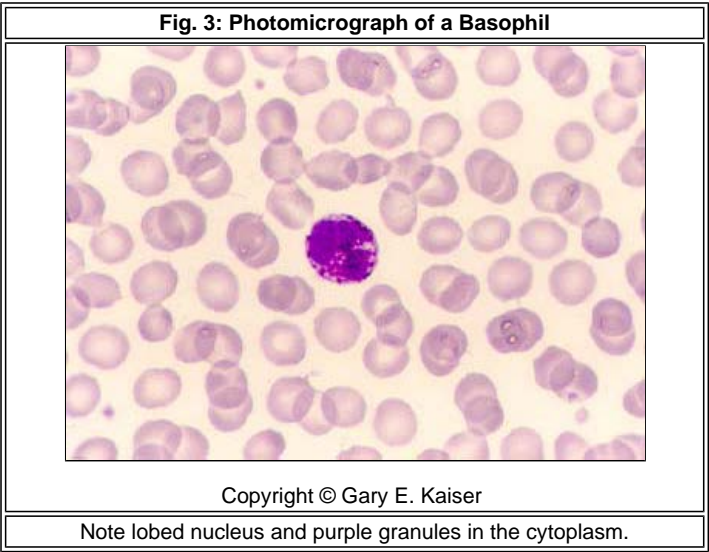
e. Their life span is 8-12 days.

Electron micrograph of an eosinophil, from the University of Illinois College of Medicine.

Transmission electron micrograph of an eosinophil from sciencephotogallery.com.

3. basophils

Basophils normally make up 0-1% of the WBCs (25-100/mm³ of blood). They are called basophils because their granules stain a dark purplish blue with the basic dye methylene blue, one of the dyes that are used when staining leukocytes (**see Fig. 3**). Basophils have a lobed nucleus.



- a. Basophils release **histamine**, **leukotrienes**, and **prostaglandins**, chemicals that **promotes inflammation** by causing vasodilation, increasing capillary permeability, and increasing mucous production. Basophils also produce heparin, platelet-activating factor (PAF), and the cytokine IL-4.
- b. Their life span is probably a few hours to a few days.

Electron micrograph of a basophil, from the Web page for the Practical Pathology of Chest Disease.

Transmission electron micrograph of an basophil from sciencephotogallery.com.

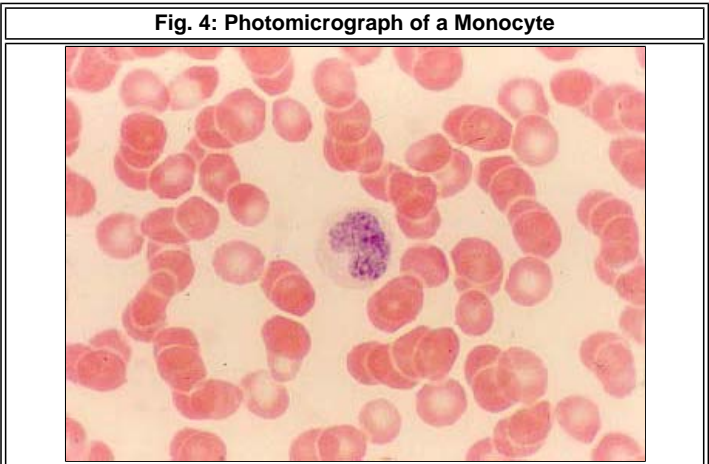
For more information: Preview of Inflammation

b. Mononuclear leukocytes (agranulocytes)

Mononuclear leukocytes (agranulocytes) have **compact nuclei** and have **no visible cytoplasmic granules**. The following are agranulocytes:

1. monocytes

Monocytes normally make up 2-8% of the WBCs (100-500/mm³ of blood). They have a compact nucleus and have no visible cytoplasmic granules (**see Fig. 4**).



Copyright © Gary E. Kaiser
Note compact nucleus and no visible cytoplasmic granules.

- a. Monocytes are important **phagocytes**.
- b. **Monocytes differentiate into macrophages and dendritic cells** when they leave the blood and enter the tissue. Macrophages and dendritic cells are very **important in phagocytosis and serve as antigen-presenting cells in the adaptive immune responses** (see below). They produce a variety of cytokines that play numerous roles in body defense.
- c. They are long-lived (life span of months) and can multiply.

Electron micrograph of a monocyte, from the Web page for the University of Illinois College of Medicine.

Transmission electron micrograph of a monocyte, from sciencephotogallery.com.

2. lymphocytes

Lymphocytes normally represent 25-40% of the WBCs (1,500-4,500/mm³ of blood).

- a. Lymphocytes **mediate the adaptive immune responses** (Unit 6).
- b. Only a small proportion of the body's lymphocytes are found in the blood. The majority are found in lymphoid tissue. In fact the collective mass of all the lymphocytes in the human body is about the same as the mass of the brain!
- c. Lymphocytes **circulate back and forth between the blood and the lymphoid system** of the body.
- d. They have a life span of days to years.
- e. There are 3 major populations of lymphocytes:

1. **B-lymphocytes (B-cells)** mediate **humoral immunity**, the production of antibody molecules against a specific antigen, and have B-cell receptors (BCR) on their surface for antigen recognition. Generally 10-20% of the lymphocytes are B-lymphocytes. Once activated, most B-lymphocytes differentiate into antibody-secreting **plasma cells**.

2. **T-lymphocytes (T-cells)** are responsible for **cell-mediated immunity**, the production of cytotoxic T-lymphocytes (CTLs), activated macrophages, activated NK cells, and cytokines against a specific antigen. They also **regulate the adaptive immune responses**. Generally 60-80% of the lymphocytes are T-lymphocytes. Based on biochemical markers on their surface, there are two major classes of T-lymphocytes:

- a. T4-lymphocytes (CD4⁺ T-lymphocytes) have **CD4 molecules** and **T-cell receptors (TCRs)** on their surface for protein antigen recognition. They function to **regulate the adaptive immune responses through cytokine production**. Once activated, they differentiate into effector T4-lymphocytes such as Th1 cells, Th2 cells, and Th17 cells.
- b. T8-lymphocytes (CD8⁺ T-lymphocytes) have **CD8 molecules** and **T-cell receptors (TCRs)** on their surface for protein antigen recognition. Once activated, they **differentiate into cytotoxic T-lymphocytes (CTLs)**.
- c. **Invariant natural killer T (iNKT) cells** are a subset of lymphocytes that bridge the gap between innate and adaptive immunity. They have **T-cell receptors (TCRs)** on their surface for glycolipid antigen recognition. Through the cytokines they produce once activated, iNKT cells are essential in both innate and adaptive immune protection against pathogens and tumors. They also play a regulatory role in the development of autoimmune diseases and transplantation tolerance.

3. **NK cells** (natural killer cells) are lymphocytes that lack B-cell receptors and T-cell receptors. They function to kill infected cells and tumor cells. NK cells are able to **kill cells to which antibody molecules have attached** through a process called antibody-dependent cellular cytotoxicity (ADCC). They also **kill human cells lacking MHC-I molecules** on their surface.

Lymphocytes will be discussed in greater detail in Unit 6.

Electron micrograph of a lymphocyte, from the Web page for the University of Illinois College of Medicine.

Transmission electron micrograph of a lymphocyte, from sciencephotogallery.com.

For more information: Preview of B-Lymphocytes
For more information: Preview of T4-Lymphocytes

For more information: Preview of T8-Lymphocytes

Although not white blood cells, **platelets** (thrombocytes) are another formed element in the blood. They promote **clotting** by sticking together after becoming activated and forming **platelet plugs** to close up damaged capillaries. They also secrete cytokines and chemokines to promote inflammation.

Electron micrograph of a platelet, from the Web page for the University of Illinois College of Medicine.

TPS Questions

Concept Map for Defense Cells in the Blood: Leukocytes

To learn more about the development of the various blood cells from hematopoietic stem cells of the bone marrow.

From the web page for the University of Illinois Association for Computing Machinery.

Self Quiz for Defense Cells in the Blood: The Leukocytes

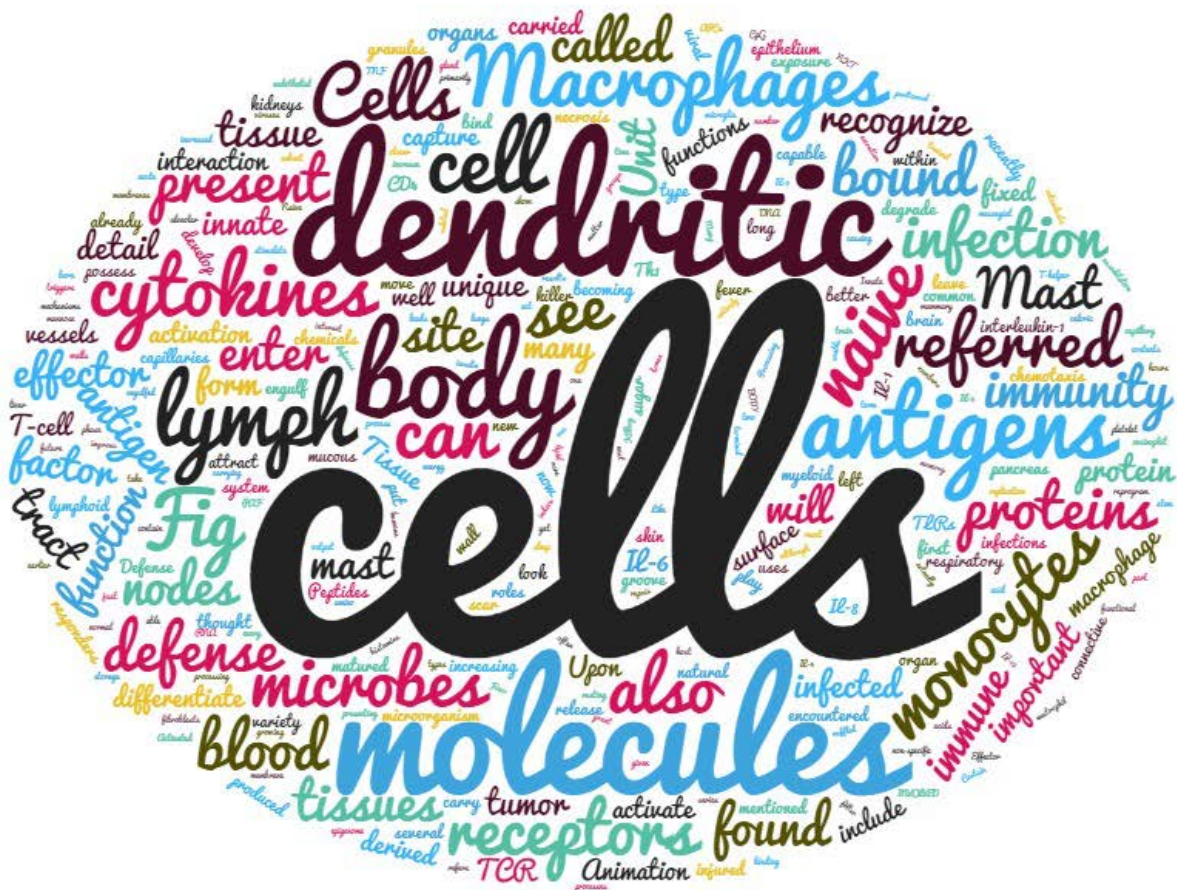
Quiz Group

Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

Innate Immunity

Defense Cells in the Tissues: Dendritic Cells, Macrophages, and Mast Cells



1. Most dendritic cells are derived from monocytes and are referred to as myeloid dendritic cells and are located throughout the epithelium of the skin, the respiratory tract, and the gastrointestinal tract, as well as lymphoid tissues and organ parenchyma.
2. Upon capturing antigens through pinocytosis and, the dendritic cells detach from their initial site, enter lymph vessels, and are carried to regional lymph nodes where they present antigens to the ever changing populations of naive T-lymphocytes.
3. The primary function of dendritic cells is to capture and present protein antigens to naive T-lymphocytes.
4. When monocytes leave the blood and enter the tissue, many become activated and differentiate into macrophages. These macrophages that have recently left the blood during inflammation and move to the site of infection through positive chemotaxis are sometimes referred to as wandering macrophages.
5. The body has macrophages already stationed throughout the tissues and organs of the body and these are sometimes referred to as fixed macrophages.
6. Functions of macrophages include killing of microbes, infected cells, and tumor cells by phagocytosis, processing antigens so they can be recognized by effector T-lymphocytes during the adaptive immune responses, and secreting mediators of inflammation such as leukotrienes, prostaglandins, and platelet-activating factor, and cytokines.
7. Mast cells are typically the immunological first responders to infection and carry out many of the same inflammatory-mediating functions as basophils.

Common Course Objectives

- 1. Explain the role of macrophages, dendritic cells, and mast cells in immunity.

Detailed Learning Objectives

- 1*.State 3 different functions of macrophages in body defense.
- 2*. State the primary function of dendritic cells in body defense.
- 3*. Name the cells in the tissue whose primary function is to present antigen to naive T-lymphocytes.
- 4*. Name the cells in the tissue whose primary function is to present antigen to effector T-lymphocytes.
- 5*. State the primary function of mast cells in body defense.

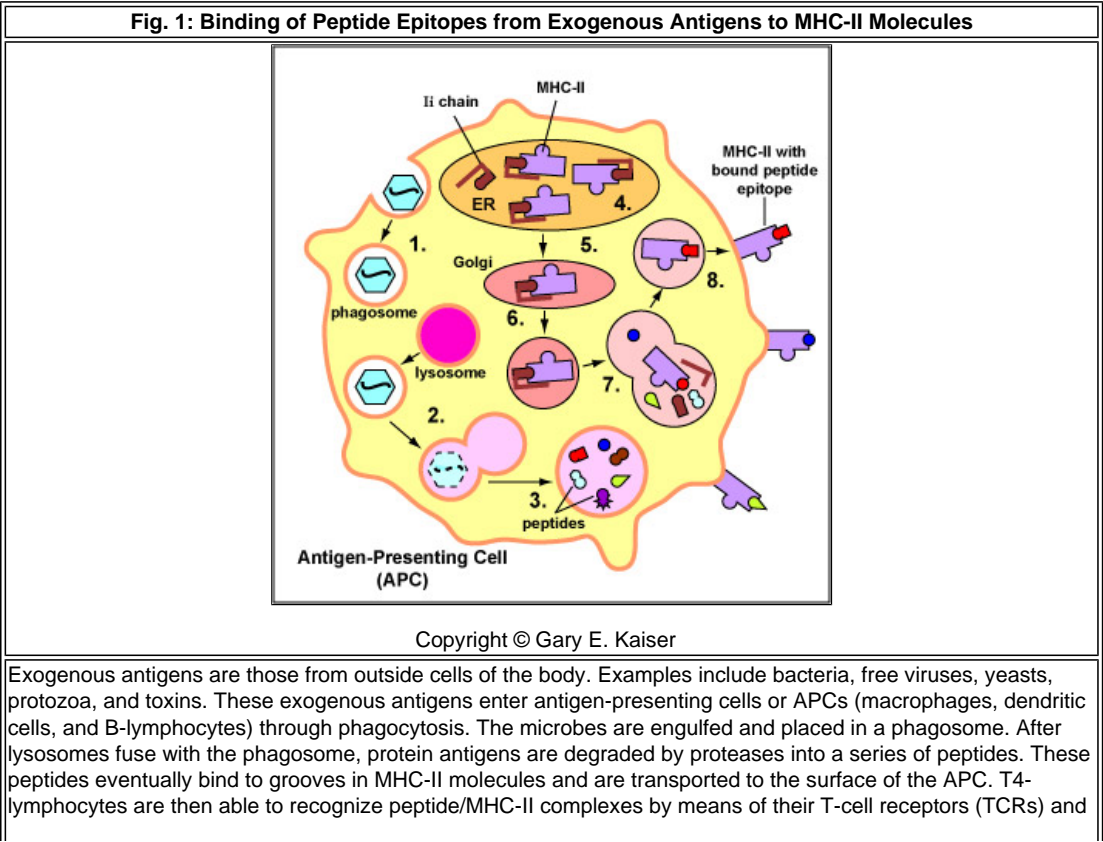
(*) = Common theme throughout the course

Defense Cells in the Tissues: Dendritic Cells, Macrophages, and Mast Cells

Dendritic Cells

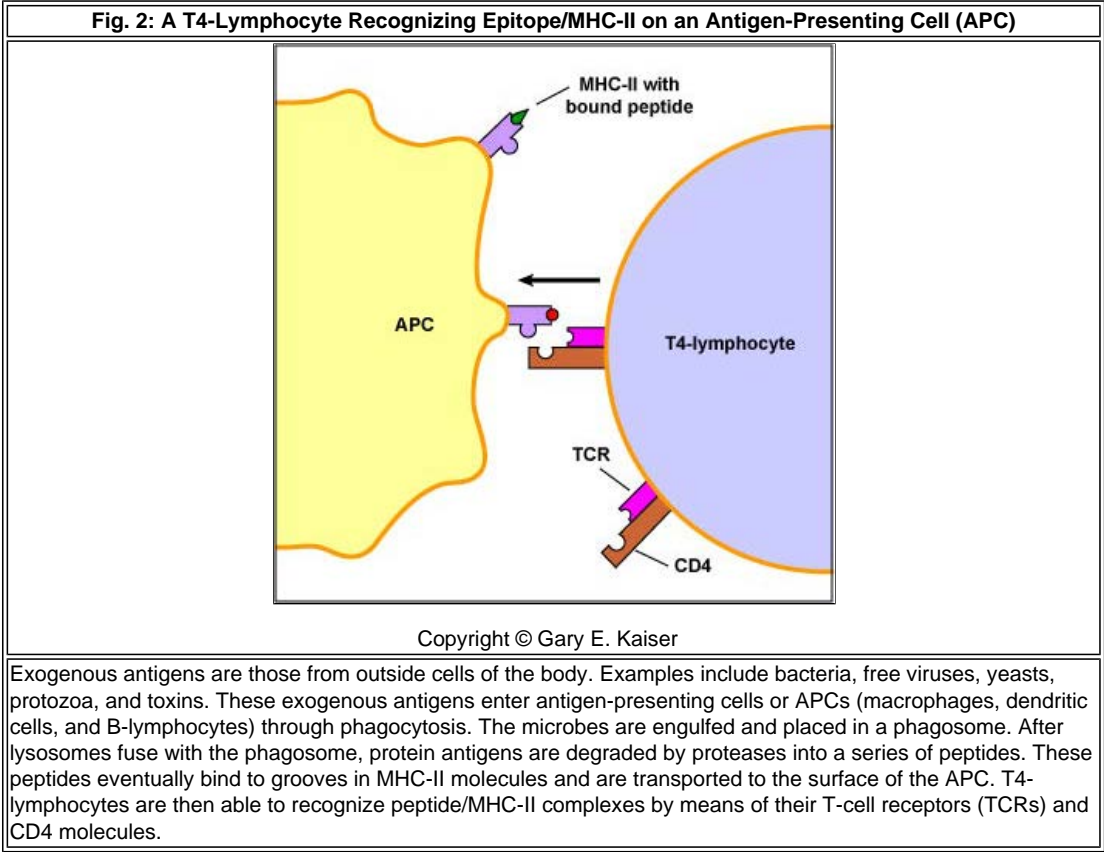
Most dendritic cells are **derived from monocytes** and are referred to as **myeloid dendritic cells**. They are **located throughout the epithelium of the skin, the respiratory tract, and the gastrointestinal tract**, as well as **lymphoid tissues and organ parenchyma**. In these locations, in their immature form, they are attached by long cytoplasmic processes. **Upon capturing antigens through pinocytosis and phagocytosis and becoming activated by inflammatory cytokines, the dendritic cells detach from their initial site, enter lymph vessels, and are carried to regional lymph nodes**. By the time they enter the lymph nodes, they have matured and are now able to **present antigen to the ever changing populations of naive T-lymphocytes** located in the cortex of the lymph nodes.

The primary function of dendritic cells is to capture and present protein antigens to naive T-lymphocytes . (Naive lymphocytes are those that have not yet encountered an antigen.) Dendritic cells engulf microorganisms and other materials and degrade them with their lysosomes. Peptides from microbial proteins are then bound to a groove of unique molecules called MHC-II molecules produced by macrophages, dendritic cells, and B-lymphocytes. The peptide epitopes bound to the MHC-II molecules are then put on the surface of the dendritic cell (**see Fig. 1**) where they can be recognized by complementary shaped T-cell receptors (TCR) and CD4 molecules on naive T4-lymphocyte (**see Fig. 2**).



CD4 molecules.

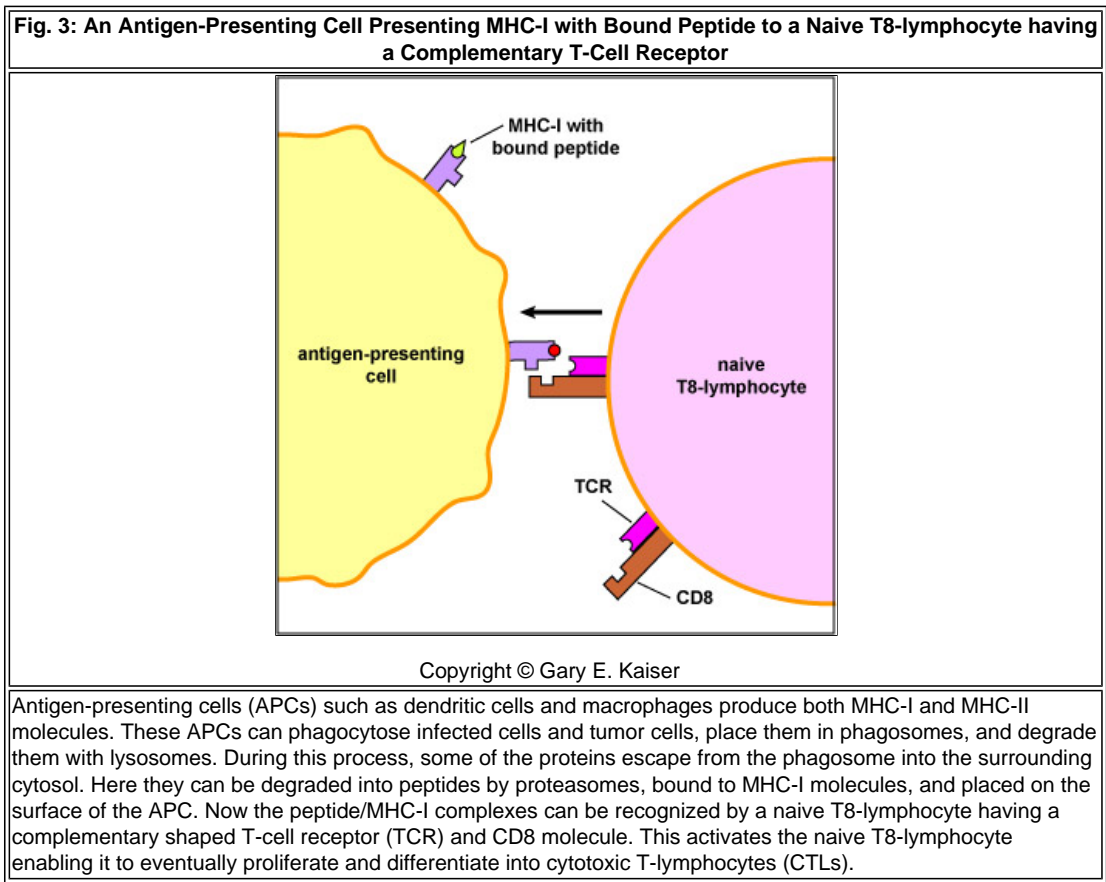
1. Exogenous antigens, such as viruses, are engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming a phagolysosome.
3. Protein antigens are degraded into a series of peptides.
4. MHC-II molecules are synthesized in the endoplasmic reticulum and transported to the Golgi complex. Once assembled, within the endoplasmic reticulum, a protein called the invariant chain (Ii) attaches to the the peptide-binding groove of the MHC-II molecules and in this way prevents peptides designated for binding to MHC-I molecules within the ER from attaching to the MHC-II.
- 5&6. The MHC-II molecules with bound Ii chain are now transported to the Golgi complex, and placed in vesicles.
7. The vesicles containing the MHC-II molecules fuse with the peptide-containing phagolysosomes. The Ii chain is removed and the peptides are now free to bind to the grooves of the MHC-II molecules.
8. The MHC-II molecules with bound peptides are transported to the cytoplasmic membrane where they become anchored. Here, the peptide and MHC-II complexes can be recognized by T4-lymphocytes by way of TCRs and CD4 molecules having a complementary shape.



Flash animation of a naive T4-lymphocyte recognizing epitopes bound to MHC-II molecules.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing epitopes reacting with a specific TCR on a T8-lymphocyte.
Naive T4-lymphocytes via the unique T-cell receptors and CD4 molecules on their surface recognize peptide epitopes from exogenous antigens bound to MHC-II molecules on antigen presenting cells (APCs). Different T-cell receptors recognize different epitopes.

For more information: Preview of antigen-presenting cells (APCs)

In addition, dendritic cells can bind peptide epitopes to MHC-I molecules and present them to naive T8-lymphocytes. The MHC-I molecules with bound peptide on the dendritic cell are recognized by complementary shaped T-cell receptors (TCR) and CD8 molecules on naive T8-lymphocyte (see Fig. 3).



Flash animation showing a naive T8-lymphocyte recognizing epitopes bound to MHC-I molecules on an APC.
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html5 version of animation for iPad showing a naive T8-lymphocyte recognizing epitopes bound to MHC-I molecules on an APC.
Naive T8-lymphocytes via the unique T-cell receptors and CD8 molecules on their surface recognize peptide epitopes from endogenous antigens bound to MHC-I molecules on antigen presenting cells (APCs). Different T-cell receptors recognize different epitopes.

Photomicrograph of a dendritic cell (Wikipedia).

Electron micrograph of a dendritic cell presenting antigen to T-lymphocytes, #1, from the Web page for the University of Illinois College of Medicine.

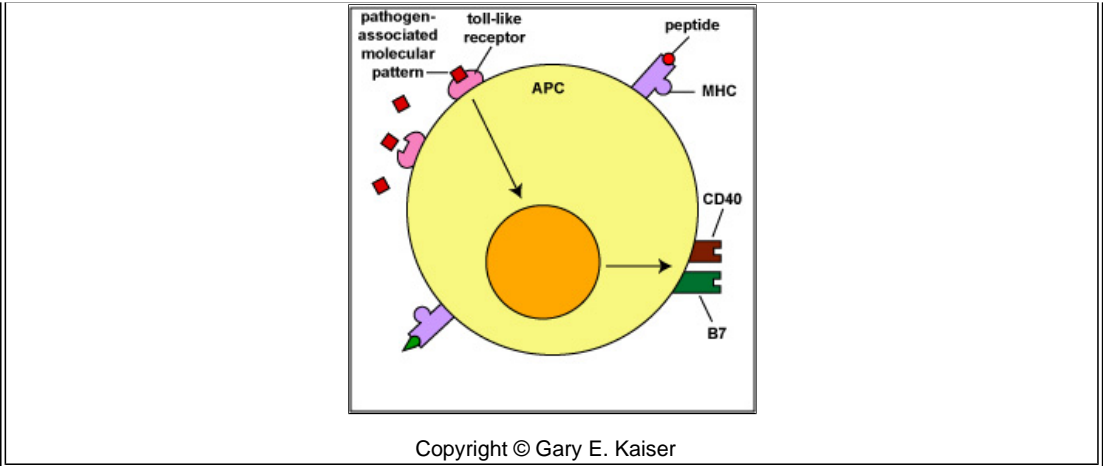
Electron micrograph of a dendritic cell presenting antigen to T-lymphocytes, #2, from the Web page for the University of Illinois College of Medicine.

These interactions enable the T4-lymphocytes or T8-lymphocytes to become activated, proliferate, and differentiate into effector cells. This will be discussed in detail in Unit 6.

- | |
|---|
| For more information: Preview of T4-lymphocytes |
| For more information: Preview of T8-lymphocytes |

Myeloid dendritic cells also use **pattern-recognition receptors** called toll-like receptors (TLRs) to **recognize pathogen-associated molecular patterns or PAMPs (see Fig. 4)**. The interaction of the PAMP with its TLR stimulates the production of co-stimulatory molecules that are also required for T-lymphocyte activation. Dendritic cells produce many of the same inflammatory cytokines as macrophages, such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8). They also can produce interleukin-12 (IL-12), a cytokine that can activate natural killer T-lymphocytes (NKT cells).

Fig. 4: Production of Co-stimulatory Molecules by Antigen-Presenting Cells (APCs)
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Antigen-presenting cells such as dendritic cells and macrophages can produce both MHC-I and MHC-II molecules. MHC-I molecules with bound peptides can be recognized by a complementary-shaped TCR/CD8 on the surface of a naive T8-lymphocyte while MHC-II molecules with bound peptides can be recognized by a complementary-shaped TCR/CD4 on the surface of a naive T4-lymphocyte. This represents the first signal necessary for activation of the naive T4- or T8-lymphocyte. Co-stimulatory signals involving the interaction of co-stimulatory molecules such as CD40 and B7 molecules on the APC with their corresponding ligands on the T4- or T8-lymphocyte are also necessary for activation. These co-stimulatory molecules are only synthesized when toll-like receptors on APCs bind to pathogen-associated molecular patterns of microbes. This is another backup system to help assure that the TCR of the lymphocyte is recognizing a nonself peptide and not a self peptide on the MHC molecules of the APC. Without the interaction of the co-stimulatory molecules, the naive T4- or T8-lymphocyte is not activated and undergoes apoptosis.

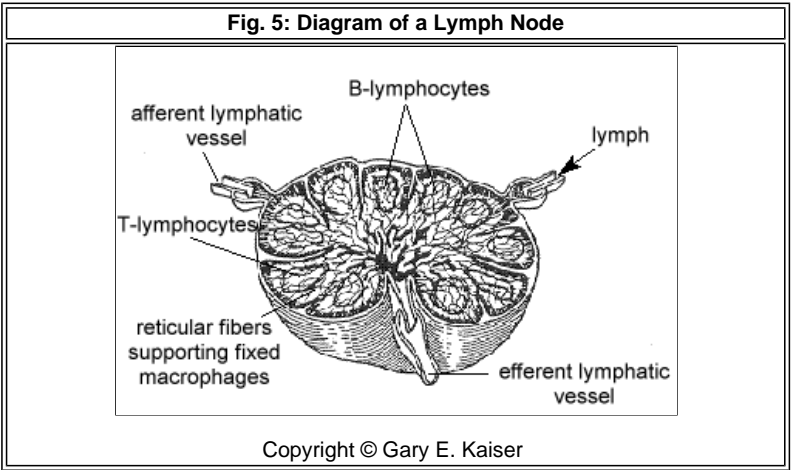
Another type of dendritic cell, the **plasmacytoid dendritic cell**, uses its TLRs to recognize viral PAMPs. This interaction results in the production and secretion of **antiviral type-I interferons**.

Macrophages

When monocytes leave the blood and enter the tissue, they become activated and differentiate into macrophages. Those that have recently left the blood during inflammation and move to the site of infection through positive chemotaxis are sometimes referred to as **wandering macrophages**.

In addition, the body has macrophages already stationed throughout all tissues and organs of the body. These are sometimes referred to as **fixed macrophages**.

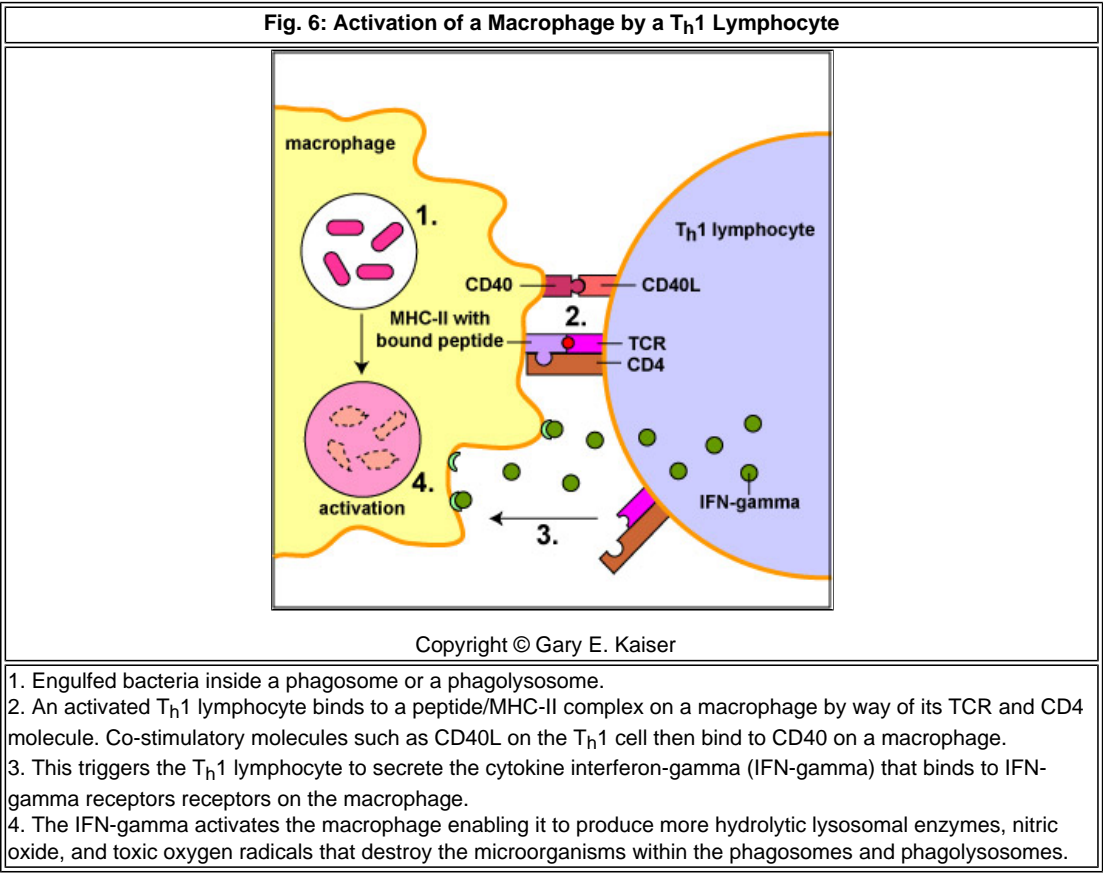
Many fixed **macrophages are part of the mononuclear phagocytic (reticuloendothelial) system**. They, along with B-lymphocytes and T-lymphocytes, are found supported by reticular fibers in lymph nodules, lymph nodes (**see Fig. 5**), and the spleen where they **filter out and phagocytose foreign matter such as microbes**. Similar cells are also found in the liver (Kupffer cells), the kidneys (mesangial cells), the brain (microglia), the bones (osteoclasts), the lungs (alveolar macrophages), and the gastrointestinal tract (peritoneal macrophages).



Macrophages actually have a number of very important functions in body defense including:

1. Killing of microbes, infected cells, and tumor cells by phagocytosis.

Macrophages that have engulfed microorganisms become activated by a subset of T-helper lymphocytes called T_h1 cells (see Fig. 6). Activated macrophages develop a ruffled cytoplasmic membrane and produce increased numbers of lysosomes.



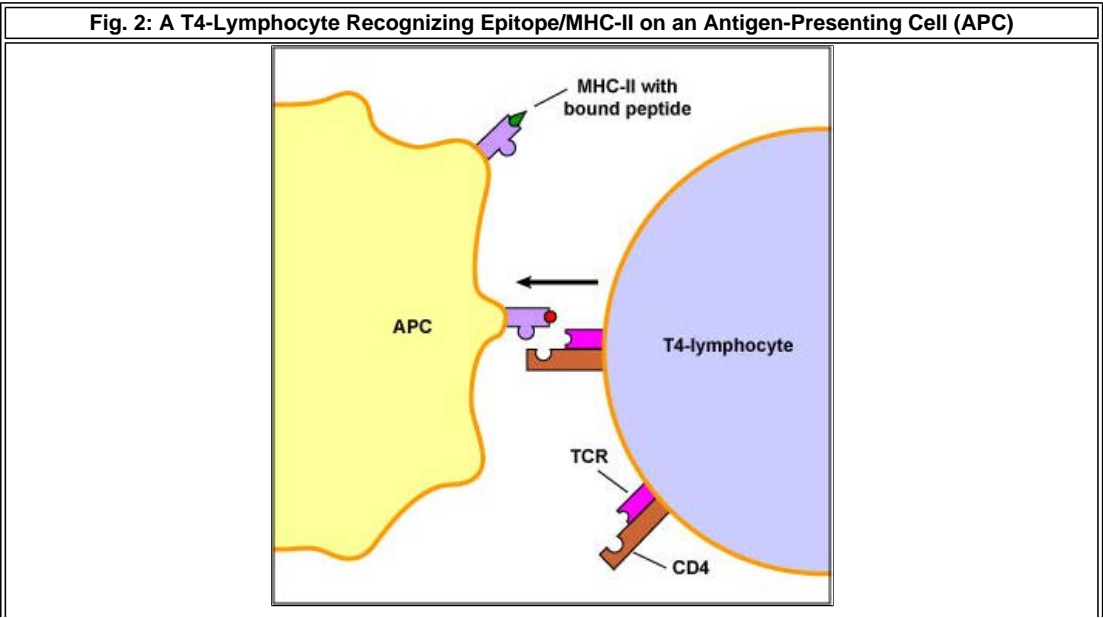
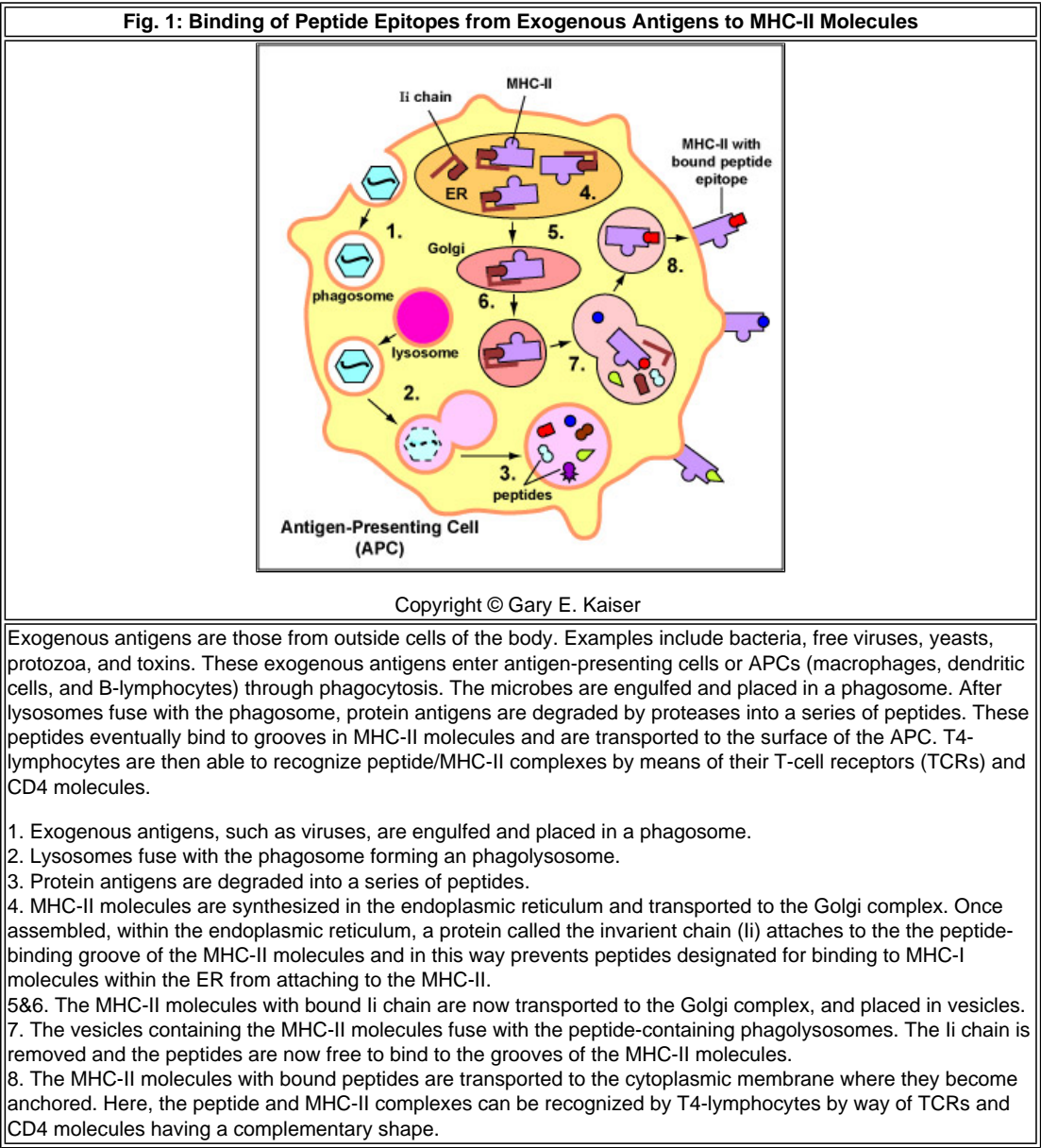
Flash animation of the activation of a macrophage by a Th1 cell.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing epitopes reacting with a specific TCR on a T8-lymphocyte.
A major function of T _h 1 cells is to both promote phagocytosis of microbes and the killing of intracellular microbes. 1. Bacteria are engulfed by a macrophage and placed in a phagosome. A lysosome fuses with the phagosome forming a phagolysosome. 2. An activated T _h 1 lymphocyte binds to a peptide/MHC-II complex on a macrophage by way of its TCR and CD4 molecule. 3. Co-stimulatory molecules such as CD40L on the T _h 1 cell then bind to CD40 on a macrophage. 4. The T _h 1 lymphocyte secretes the cytokine interferon-gamma (IFN-gamma) that binds to IFN-gamma receptors receptors on the macrophage. 5. The IFN-gamma activates the macrophage enabling it to produce more hydrolytic lysosomal enzymes, nitric oxide, and toxic oxygen radicals that destroy the microorganisms within the phagosomes and phagolysosomes.

2. Processing antigens so they can be recognized by effector T-lymphocytes during the adaptive immune responses.

Macrophages, as well as the dendritic cells mentioned below, process antigens through phagocytosis and present them to T-lymphocytes. Because of this function, they are often referred to as antigen-presenting cells or APCs .

Macrophages primarily capture and present protein antigens to effector T-lymphocytes. (Effector lymphocytes are lymphocytes that have encountered an antigen, proliferated, and matured into a form capable of actively carrying out immune defenses.) Macrophages engulf the microorganism and degrade it with their lysosomes. Peptides from microbial proteins are then bound to a groove of unique molecules called MHC-II molecules produced by macrophages, dendritic cells, and B-lymphocytes. The peptide epitopes bound to the MHC-II molecules are then put on the surface of the macrophage (see Fig. 1) where they can be recognized by complementary shaped T-cell receptors (TCR) and CD4 molecules on an effector T4-lymphocyte (see Fig. 2). This interaction leads to the

activation of that macrophage. This will be discussed in detail in Unit 6.



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Exogenous antigens are those from outside cells of the body. Examples include bacteria, free viruses, yeasts, protozoa, and toxins. These exogenous antigens enter antigen-presenting cells or APCs (macrophages, dendritic cells, and B-lymphocytes) through phagocytosis. The microbes are engulfed and placed in a phagosome. After lysosomes fuse with the phagosome, protein antigens are degraded by proteases into a series of peptides. These peptides eventually bind to grooves in MHC-II molecules and are transported to the surface of the APC. T4-lymphocytes are then able to recognize peptide/MHC-II complexes by means of their T-cell receptors (TCRs) and CD4 molecules.

Like dendritic cells discussed above, macrophages are also capable of capturing and presenting protein antigens to naive T-lymphocytes although they are not as important in this function.

Photomicrograph of a macrophage (Wikipedia)

Scanning electron micrographs of a macrophage with pseudopods and a macrophage phagocytosis *E. coli* on a blood vessel; courtesy of Dennis Kunkel's Microscopy.

Scanning electron micrograph of a macrophage presenting antigen to a T-lymphocyte, from the Cells Alive Web Page.

3. **Secreting lipid mediators of inflammation such as leukotrienes, prostaglandins, and platelet-activating factor (PAF).**

4. **Secreting proteins called cytokines that play a variety of roles in non-specific body defense.**

Macrophage-produced cytokines promote inflammation and induce fever, increase phagocytosis and energy output, promote sleep, activate resting T-lymphocytes, attract and activate neutrophils, and stimulate the replication of endothelial cells to form capillaries and fibroblasts to form connective scar tissue. Four important cytokines that macrophages produce are **tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8)**. Cytokines will be discussed in more detail in Unit 4 and Unit 5.

For more information: Preview of cytokines

For more information: Preview of inflammation

There is growing evidence that **monocytes and macrophages can be "trained" by an earlier infection to do better in future infections, that is, develop memory**. It is thought that microbial pathogen-associated molecular patterns (PAMPs) binding to pattern-recognition (PRRs) on monocytes and macrophages triggers the cell's epigenome to reprogram or train that cell to react better against new infections.

Macrophages show great functional diversity. In addition to the populations of macrophages involved in body defense and immunity, there are populations of macrophages that play important roles in:

1. The development of a variety of tissues and organs within the body, including the brain, blood cells, mammary gland, pancreas, and kidneys.
2. Modulating normal physiology and maintaining homeostasis in the body, including insulin resistance and sensitivity, long term nutrient storage, thermo genesis, and liver and pancreas function in response to caloric intake.
3. Tissue repair, including the formation of scar tissue and the growth of new capillaries into injured tissues.

Mast Cells

Mast cells are typically the immunological first responders to infection and carry out many of the same inflammatory-mediating functions as basophils. There are two types of mast cells in the body: mast cells found in the **connective tissue** and mast cells found throughout the mucous membranes.

The granules of mast cells contain such mediators as histamine, eosinophil chemotactic factor, neutrophil chemotactic factor, platelet activating factor, and cytokines such as IL-3, IL-4, IL-5, IL-6, and TNF-alpha. They also possess pathways for synthesizing leukotrienes and prostaglandins, chemicals that **promote inflammation** by causing vasodilation, increasing capillary permeability, and increasing mucous production.

Mast cells have pattern-recognition receptors or PRRs on their surface that interact with pathogen-associated molecular patterns or PAMPs of microbes. After the PAMPs bind to their respective PRRs, they release the contents of their granules. These chemical mediators promote inflammation and attract neutrophils to the infected site.

For more information: Preview of inflammation

For more information: Preview of pathogen-associated molecular

patterns (PAMPs)
For more information: Preview of pattern-recognition receptors (PRRs)

Photomicrograph of a mast cell (Wikipedia)

Electron micrograph of a mast cell, from www.nhs.uk

Concept Map for Defense Cells in the Tissues: Dendritic Cells, Macrophages, and Mast Cells
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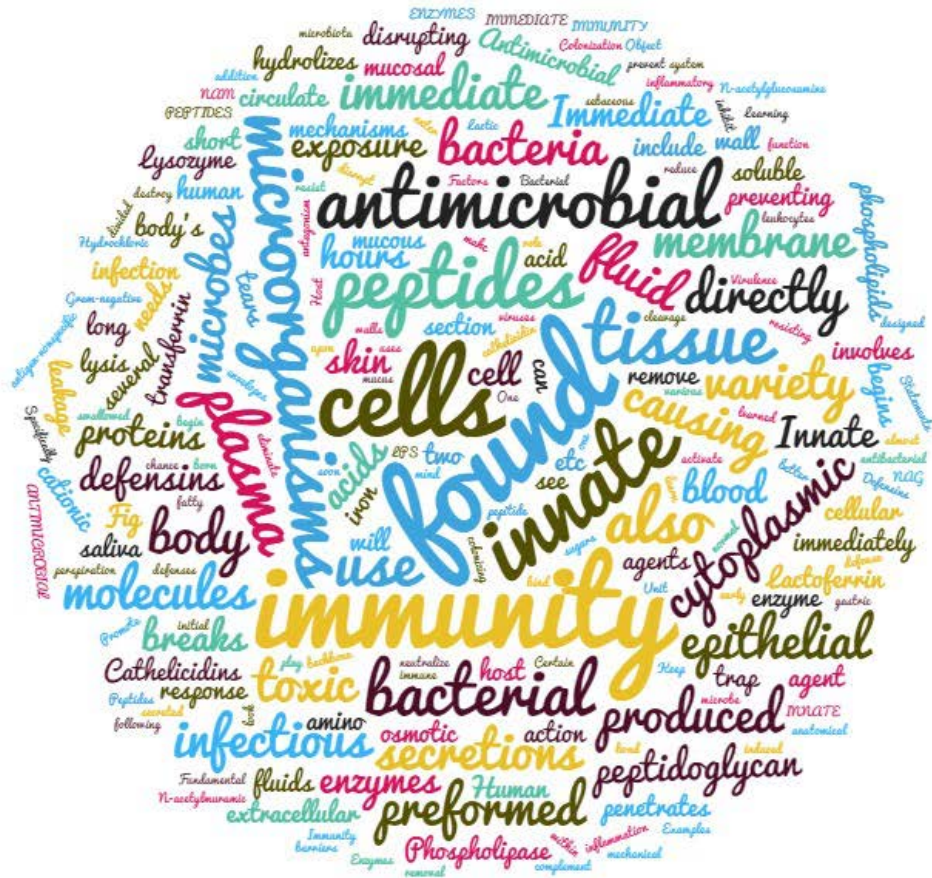
Self Quiz for Defense Cells in the Tissues: Dendritic Cells, Macrophages, and Mast Cells

Quiz Group

Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

Immediate Innate Immunity: Antimicrobial Enzymes and Peptides



1. Immediate innate immunity begins 0-4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood and are found in extracellular tissue fluids.
2. Lysozyme, found in tears, mucous, saliva, plasma, tissue fluid, etc., breaks down peptidoglycan in bacteria causing osmotic lysis.
3. Phospholipase A2 is an enzyme that penetrates the bacterial cell wall and hydrolyzes the phospholipids in the bacterial cytoplasmic membrane.
4. Human defensins are short cationic peptides 30-40 amino acids long that are directly toxic by disrupting the cytoplasmic membrane of a variety of microorganisms causing leakage of cellular needs.
5. Cathelicidins are proteins produced by skin and mucosal epithelial cells that are directly toxic to a variety of microorganisms.
6. Lactoferrin and transferrin, found in body secretions, plasma, and tissue fluid, trap iron for use by human cells while preventing its use by microorganisms.

1. State how long it takes for immediate innate immunity to become activated and what it involves.
- 2*. State the function of the following antimicrobial enzymes and peptides:

- a. lysozyme
- b. phospholipase A₂
- c. defensins
- d. cathelicidins
- e. lactotransferrin and transferrin

(*) = Common theme throughout the course

Immediate Innate Immunity: Antimicrobial Enzymes and Peptides

Immediate Innate Immunity

Immediate innate immunity begins 0-4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood, our found in extracellular tissue fluids, and are secreted by epithelial cells. These include:

- antimicrobial enzymes and peptides;
- complement system proteins; and

These preformed antimicrobial molecules are designed to immediately begin to remove infectious agents as soon as they enter the body.

In addition to preformed antimicrobial molecules, the following also play a role in immediate innate immunity:

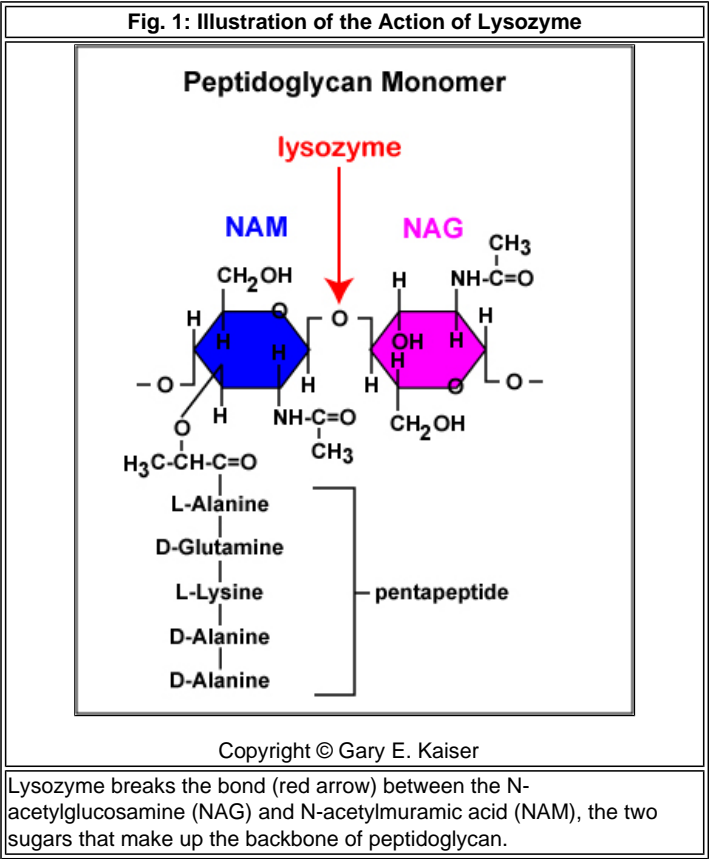
- anatomical barriers to infection
- mechanical removal of microbes
- bacterial antagonism by the body's normal microbiota

In this section we will look at how antimicrobial enzymes and peptides function to remove infectious agents.

Antimicrobial Enzymes and Antimicrobial Peptides

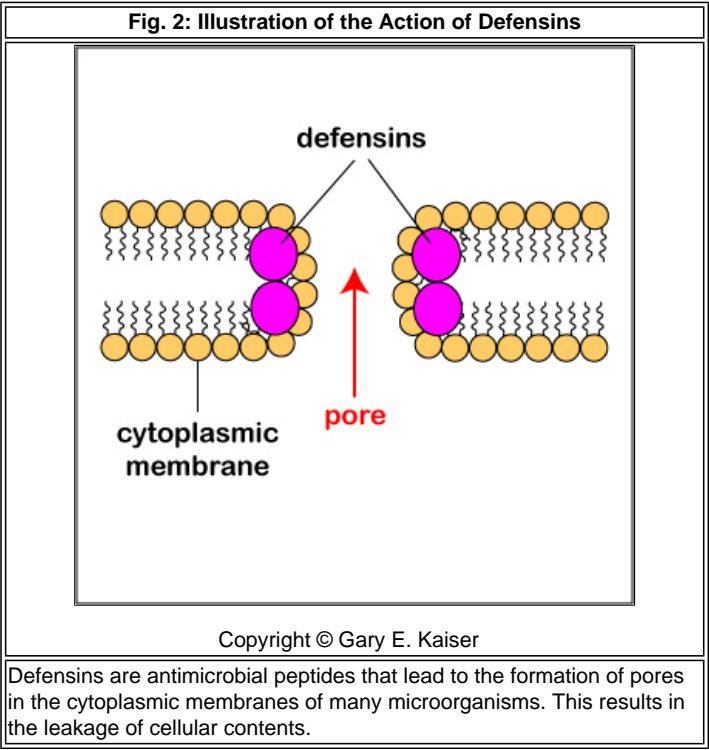
Examples include:

- a. **Lysozyme**, found in in tears, mucous, saliva, plasma, tissue fluid, etc., breaks down peptidoglycan in bacteria causing osmotic lysis. Specifically, it breaks the bond between the N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), the two sugars that make up the backbone of peptidoglycan (**see Fig. 1**).



- b. **Phospholipase A₂** is an enzyme that penetrates the bacterial cell wall and **hydrolyzes the phospholipids in the bacterial cytoplasmic membrane**.
- c. **Human defensins** are short cationic peptides 30-40 amino acids long that are **directly toxic by disrupting the cytoplasmic membrane of a variety of**

microorganisms causing leakage of cellular needs (see Fig. 2). They also **activate cells for an inflammatory response**. Defensins are produced by leukocytes, epithelial cells, and other cells. They are also found in blood plasma and mucus. Certain defensins also disrupt the envelopes of some viruses.



- d. **Cathelicidins** are proteins produced by skin and mucosal epithelial cells. The two peptides produced upon cleavage of the cathelicidin are **directly toxic to a variety of microorganisms**. One peptide also can bind to and neutralize LPS from Gram-negative cell walls to reduce inflammation.
- e. **Lactic and fatty acids**, found in perspiration and sebaceous secretions, **inhibit microbes on the skin**.
- f. **Lactoferrin and transferrin**, found in body secretions, plasma, and tissue fluid, **trap iron for use by human cells while preventing its use by microorganisms**.
- g. **Hydrochloric acid** and enzymes found in gastric secretions **destroy microbes that are swallowed**.

Keep in mind that in Unit 3 under "Virulence Factors that Promote Bacterial Colonization of the Host" we learned several mechanisms that various bacteria use to resist the body's antibacterial peptides. By resisting these immediate innate immune defenses, some bacteria have a better chance of colonizing their host.

Concept Map for Antibacterial Enzymes and Peptides

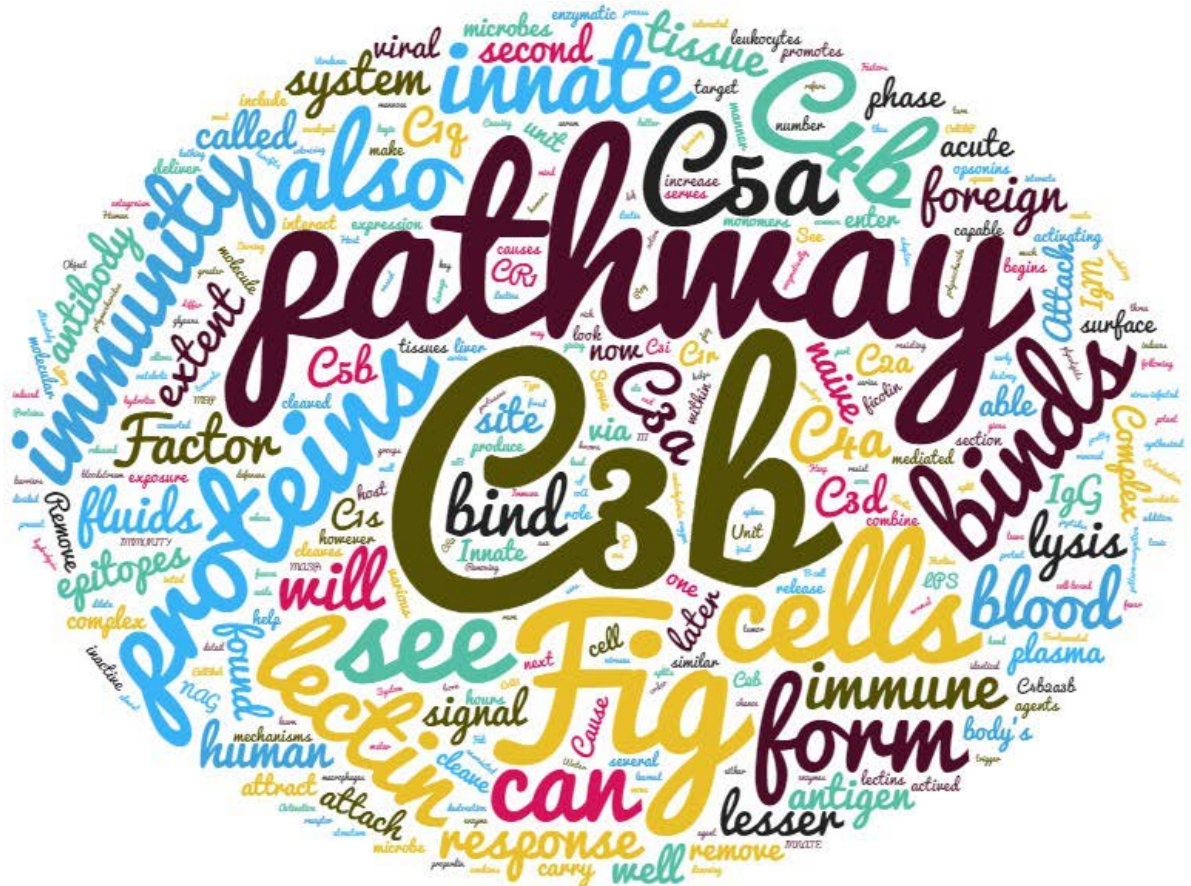
[Quiz Group](#)

[Return to Unit 5 and 6 Table of Contents](#)

[Back to Softchalk Lessons Table of Contents](#)

Innate Immunity

Immediate Innate Immunity: The Complement Pathways



Fundamental Statement for this Softchalk Lesson:

1. The proteins of the complement system circulate in an inactive form, but in response to the recognition of molecular components of microorganism, they become sequentially activated, working in a cascade where in the binding of one protein promotes the binding of the next protein in the cascade.
2. There are 3 complement pathways that make up the complement system: the classical complement pathway, the lectin pathway, and the alternative complement pathway.
3. The classical complement pathway is initiated by activation of C1. C1 is primarily activated by interacting with the Fc portion of the antibody molecules IgG or IgM after they have bound to their specific antigen. C1 is also able to directly bind to the surfaces of some pathogens as well as with the C-reactive protein (CRP) that is produced during the acute phase response of innate immunity.
4. The lectin pathway is activated by the interaction of microbial carbohydrates (lectins) with mannose-binding lectin (MBL) or ficolins found in the plasma and tissue fluids.
5. The alternative complement pathway is activated by C3b binding to microbial surfaces and to antibody molecules.
7. All complement pathways carry out the same 6 beneficial innate defense functions.
8. The complement proteins C5a and, to a lesser extent, C3a, and C4a trigger vasodilation and inflammation in order to deliver defense cells and defense chemicals to the infection site.
9. The complement protein C5a also functions as a chemoattractant for phagocytes.
10. The complement proteins C3b and to a lesser extent, C4b can function as opsonins, that is, they can attach antigens to phagocytes.
11. The complement proteins C5b6789n, functions as a Membrane Attack Complex (MAC) causing lysis of Gram-negative bacteria, human cells displaying foreign epitopes, and viral envelopes.
12. The complement protein C3d serves as a second signal for activating naive B-lymphocytes during adaptive immunity.
13. The complement proteins C3b and to a lesser extent, C4b help to remove harmful immune complexes from the body.

Common Course Objective

- 1. Recall the three ways complement can be activated and describe the beneficial roles the complement pathways play in innate immunity.

Detailed Learning Objectives

- 1*. Briefly describe how the classical complement pathway is activated. 2*. Briefly describe the beneficial effects of the following complement pathway products:
 - a. C5a
 - b. C3a
 - c. C3b
 - d. C4b
 - e. C3d
 - f. C5b6789_n (MAC)
 - 3*. Briefly describe how the lectin pathway is activated.
 - 4*. Briefly describe how the alternative complement pathway is activated.
- (*) = Common theme throughout the course

TPS Questions

Immediate Innate Immunity: The Complement Pathways

Immediate Innate Immunity

Immediate innate immunity begins 0-4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood, our found in extracellular tissue fluids, and are secreted by epithelial cells. These include:

- antimicrobial enzymes and peptides;
- complement system proteins; and

These preformed antimicrobial molecules are designed to immediately begin to remove infectious agents as soon as they enter the body.

In addition to preformed antimicrobial molecules, the following also play a role in immediate innate immunity:

- anatomical barriers to infection
- mechanical removal of microbes
- bacterial antagonism by the body's normal microbiota

In this section we will look at how antimicrobial enzymes and peptides function to remove infectious agents.

The Complement System

The complement system refers to a series of more than 30 soluble, preformed proteins circulating in the blood and bathing the fluids surrounding tissues. The proteins circulate in an inactive form, but in response to the recognition of molecular components of microorganism, they become sequentially activated, working in a cascade where in the binding of one protein promotes the binding of the next protein in the cascade.

There are 3 complement pathways that make up the complement system: **the classical complement pathway, the lectin pathway, and the alternative complement pathway**. The pathways differ in the manner in which they are initiated and ultimately produce a key enzyme called C3 convertase:

1. The **classical complement pathway** is initiated by activation of C1. C1 is primarily activated by interacting with the Fc portion of the antibody molecules IgG or IgM after they have bound to their specific antigen. C1 is also able to directly bind to the surfaces of some pathogens as well as with the C-reactive protein (CRP) that is produced during the acute phase response of innate immunity.
2. The **lectin pathway** is activated by the **interaction of microbial carbohydrates (lectins) with mannose-binding lectin (MBL)** or ficolins found in the plasma and tissue fluids.
3. The **alternative complement pathway** is activated by **C3b binding to microbial surfaces and to antibody molecules** .

The end results and defense benefits of each pathway, however, are the same. All complement pathways carry out 6 beneficial innate defense functions. Proteins

produced by the complement pathways:

1. **Trigger inflammation;**
2. **Chemotactically attract phagocytes to the infection site;**
3. **Promote the attachment of antigens to phagocytes (enhanced attachment or opsonization);**
4. **Cause lysis of Gram-negative bacteria, human cells displaying foreign epitopes, and viral envelopes;**
5. **Play a role in the activation of naive B-lymphocytes during adaptive immunity;** and
6. **Remove harmful immune complexes from the body.**

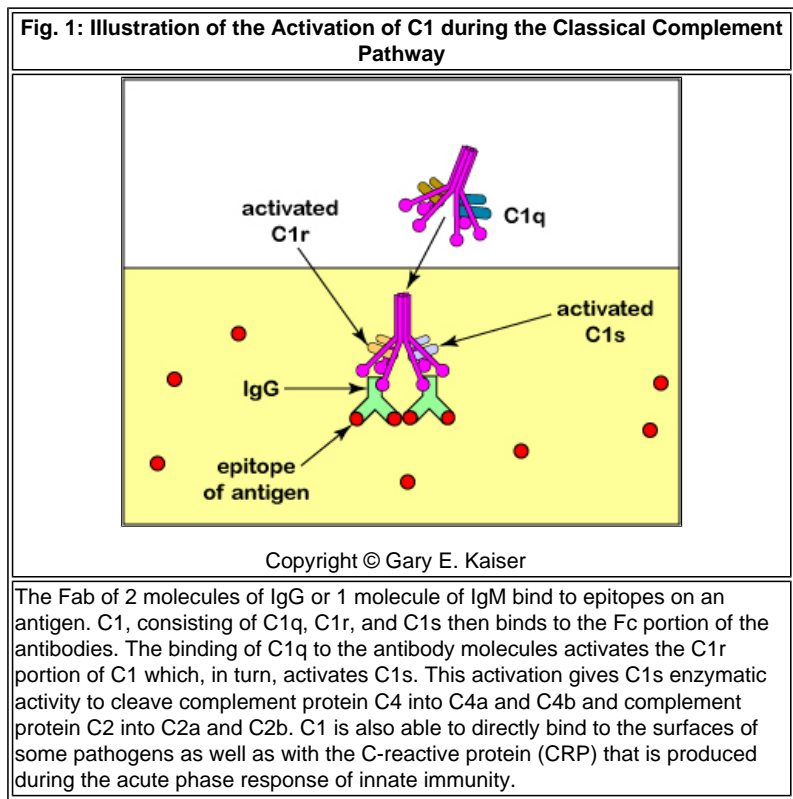
We will now look at each of these complement pathways and see how they function to protect the body.

The Classical Complement Pathway

The classical complement pathway is primarily activated when a complement protein complex called C1 interacts with the Fc portion of the antibody molecules IgG or IgM after they have bound to their specific antigen via their Fab portion. C1 is also able to directly bind to the surfaces of some pathogens as well as with the C-reactive protein (CRP) that is produced during the acute phase response of innate immunity.

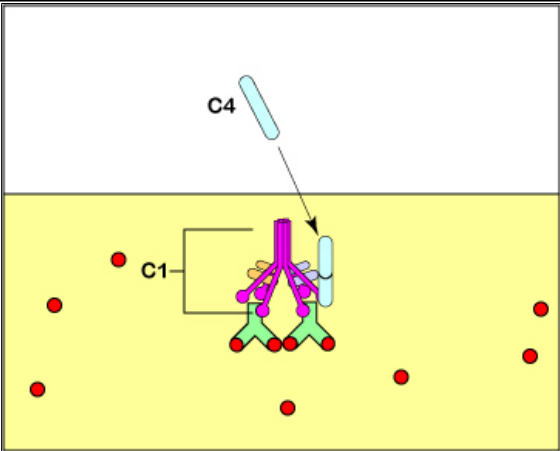
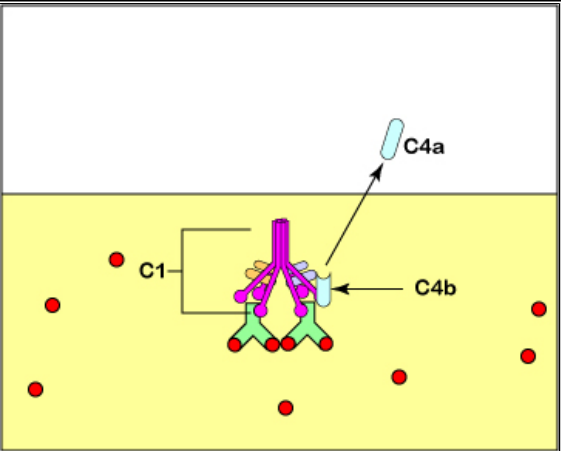
The C1 complex is composed of three complement proteins called C1q, C1r, and C1s.

1. The C1q is the portion of the C1 complex that binds to the antibodies, the microbe, or the CRP (**see Fig. 1**).

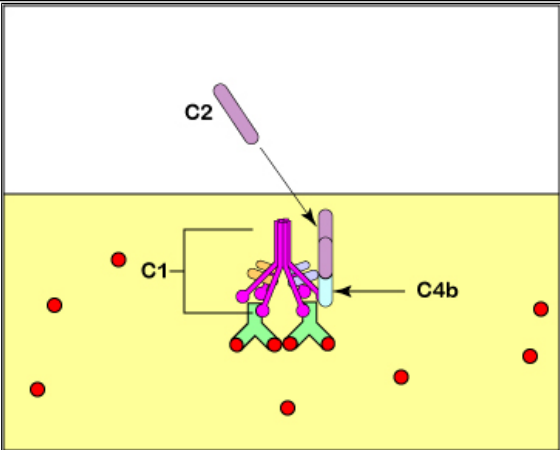
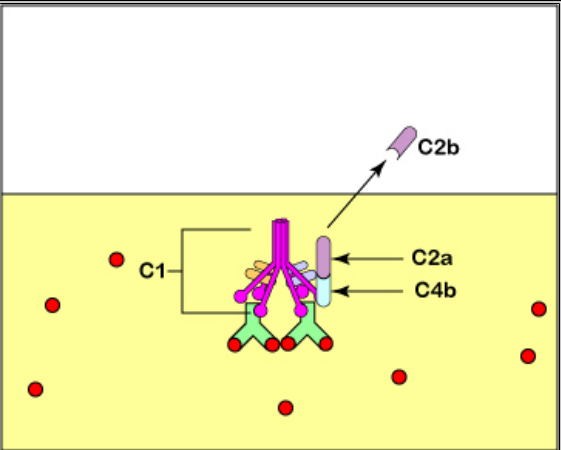


Flash animation showing assembly of C1 during the classical complement pathway.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing assembly of C1 during the classical complement pathway.
The C1 complex is composed of three complement proteins called C1q, C1r, and C1s. The C1q is the portion of the C1 complex that can bind to the Fc portion of the antibodies IgG or IgM, certain microbes, or the C-reactive protein (CRP) from the acute phase response.
In the case of antibodies, the Fab portions of 2 molecules of IgG (or 1 molecule of IgM) bind to their corresponding epitopes on an antigen. Binding of the IgG to the epitopes activates the Fc portion of the IgG enabling C1q of the C1 complex to bind. The binding of C1q to the antibody molecules activates the C1r portion of C1 which, in turn, activates C1s. This activation gives C1s enzymatic activity to cleave complement protein C4 into C4a and C4b and complement protein C2 into C2a and C2b.

2. The binding of C1q activates the C1r portion of C1 which, in turn, activates C1s. This activation gives C1s enzymatic activity to cleave complement protein C4 into C4a and C4b (**see Fig. 2A and Fig. 2B**).

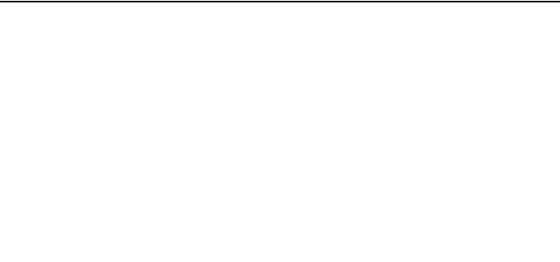
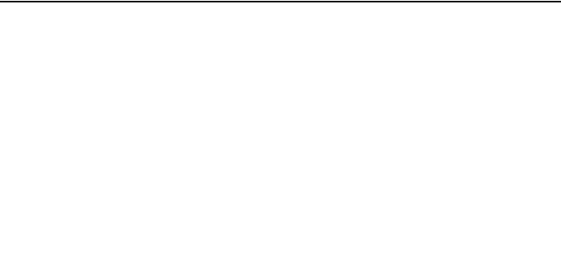
Fig. 2A: Illustration of Cleavage of C4 by C1 during the Classical Complement Pathway - Step 1	Fig. 2B: Illustration of Cleavage of C4 by C1 during the Classical Complement Pathway - Step 2
	
Copyright © Gary E. Kaiser	Copyright © Gary E. Kaiser
The enzyme C1 is able to cleave C4 into C4a and C4b. The C4b then binds to adjacent proteins and carbohydrates on the surface of the antigen	After enzyme C1 cleaves C4 into C4a and C4b. The C4b binds to adjacent proteins and carbohydrates on the surface of the antigen. C4a can weakly promote inflammation.

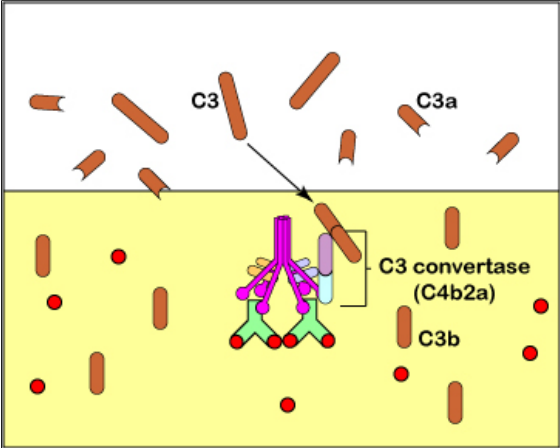
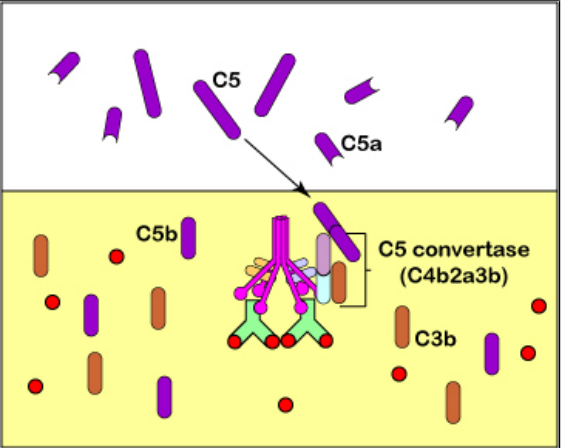
3. C2 then binds to C4b and is cleaved by C1 into C2a and C2b (see Fig. 3A and Fig. 3B).

Fig. 3A: Illustration of Cleavage of C2 by C1 during the Classical Complement Pathway - Step 1	Fig. 3B: Illustration of Cleavage of C2 by C1 during the Classical Complement Pathway - Step 2
	
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C2 binds to the C4b and the enzyme C1 subsequently cleaves C2 into C2a and C2b. The C4b2a functions as a C3 convertase that can enzymatically cleave hundreds of molecules of C3 into C3a and C3b.	C2 binds to the C4b and the enzyme C1 subsequently cleaves C2 into C2a and C2b. The C4b2a functions as a C3 convertase that can enzymatically cleave hundreds of molecules of C3 into C3a and C3b.

4. C4b and C2a combine to form **C4b2a, the C3 convertase**. C3 convertase can now **cleave hundreds of molecules of C3 into C3a and C3b** (see Fig. 4).

5. Some molecules of C3b bind to C4b2a, the C3 convertase, to form **C4b2a3b, a C5 convertase** that cleaves C5 into C5a and C5b (see Fig. 5).

Fig. 4: Illustration of Cleavage of C3 by C3 Convertase during the Classical Complement Pathway	Fig. 5: Illustration of Formation of C5 Convertase during the Classical Complement Pathway and Cleavage of C5 by C5 convertase
	

	
Copyright © Gary E. Kaiser	Copyright © Gary E. Kaiser
<p>The C4b2a functions as a C3 convertase that can enzymatically cleave hundreds of molecules of C3 into C3a and C3b. C3b, and to a lesser extent C4b, attaches antigens to phagocytes for opsonization (enhanced attachment). One portion of the C3b binds to proteins and polysaccharides on microbial surfaces; another portion binds to CR1 receptors on phagocytes, B-lymphocytes, and dendritic cells. This results in improved phagocytosis. C3a can promote inflammatory responses that enable body defense cells and defense chemicals to leave the blood and enter the tissues.</p>	<p>C4b2a3b functions as a C5 convertase that cleaves C5 into C5a and C5b. C5a is the most potent complement protein triggering inflammation. It causes capillary vasodilation and also binds to mast cells causing them to release vasodilators such as histamine to increase blood vessels permeability; it increases the expression of adhesion molecules on leukocytes and the vascular endothelium so that leukocytes can squeeze out of the blood vessels and enter the tissue (diapedesis); it causes neutrophils to release toxic oxygen radicals for extracellular killing; and it induces fever. C5a also functions as a chemoattractant for phagocytes. Leukocytes will move towards increasing concentrations of C5a. C5b becomes part of the Membrane Attack Complex (MAC).</p>

Flash animation showing formation of C3 convertase and C5 convertase during the classical complement pathway.

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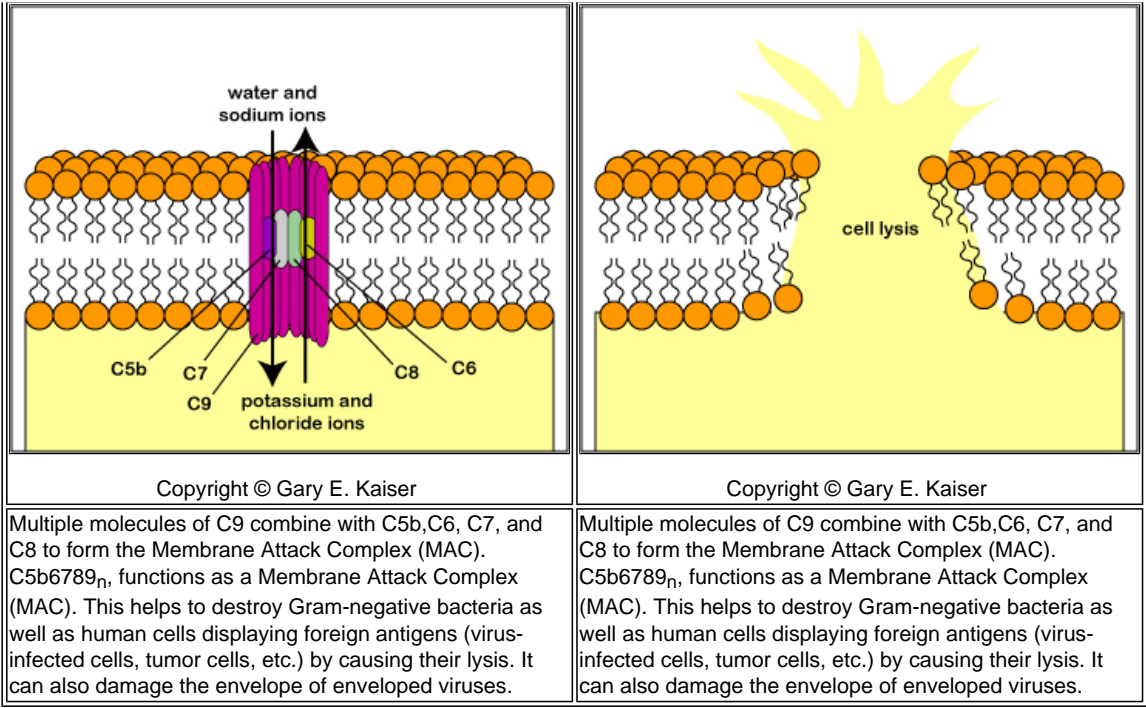
html5 version of animation for iPad showing formation of C3 convertase and C5 convertase during the classical complement pathway.

The enzyme C1 is able to cleave C4 into C4a and C4b. The C4b binds to adjacent proteins and carbohydrates on the surface of the antigen. C2 then binds to the C4b and C1 cleaves C2 into C2a and C2b. The C4b2a functions as a C3 convertase that can subsequently cleave hundreds of molecules of C3 into C3a and C3b. C3b attaches antigens to phagocytes for opsonization (enhanced attachment) while C3a can promote inflammatory responses that enable body defense cells and defense chemicals to leave the blood and enter the tissues.

Some of the C3b combines with the C4b and the C2a. C4b2a3b functions as a C5 convertase that cleaves molecules of C5 into C5a and C5b. C5a is the most potent complement protein triggering inflammation. C5b becomes part of the Membrane Attack Complex (MAC).

6. C5b binds to the surface of the target cell and subsequently binds C6, C7, C8, and a number of monomers of C9 to form **C5b6789_n**, the **Membrane Attack Complex (MAC)** (see Fig. 6 and Fig. 7).

<p>Fig. 6: Illustration of Formation of the Membrane Attack Complex (MAC) during the Complement Pathways</p>	<p>Fig. 7: Illustration of Membrane Attack Complex (MAC)-Induced Lysis during the Complement Pathways</p>



Flash animation showing the formation of the Membrane Attack Complex (MAC) and cytolysis during the complement pathways.

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html5 version of animation for iPad showing the formation of the Membrane Attack Complex (MAC) and cytolysis during the complement pathways.

This C5b6789_n, or membrane attack complex (MAC), puts pores into lipid bilayer membranes of human cells to which antibodies have bound. This results in cell lysis. MAC can also damage the envelope of enveloped viruses and put pores in the outer membrane and cytoplasmic membrane of Gram-negative bacteria causing their lysis

- For more information: Preview of antigens, epitopes, and immunogens
- For more information: Preview of antibodies

As mentioned above, proteins of the complement pathways carry out 6 beneficial innate defense functions. These include:

1. Triggering inflammation

C5a is the most potent complement protein triggering inflammation. It reacts with blood vessels causing vasodilation. It also causes **mast cells to release vasodilators** such as histamine, increasing blood vessel permeability as well as increasing the expression of adhesion molecules on leukocytes and the vascular endothelium so that **leukocytes can squeeze out of the blood vessels and enter the tissue (diapedesis)**. C5a also causes **neutrophils to release toxic oxygen radicals** for extracellular killing and induces **fever**. To a lesser extent **C3a** and **C4a** also promote inflammation. As we will see later in this unit, inflammation is a process in which blood vessels dilate and become more permeable, thus enabling body defense cells and defense chemicals to leave the blood and enter the tissues.

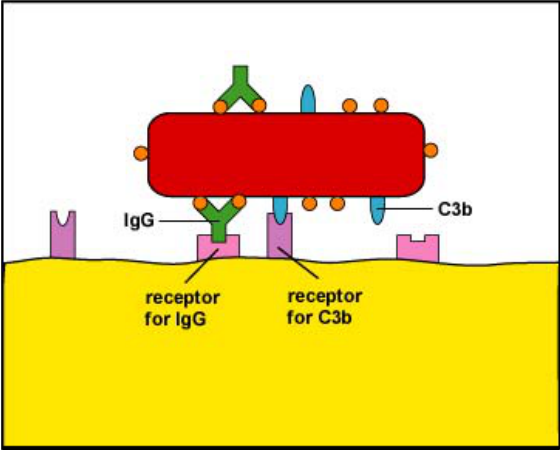
2. Chemotactically attracting phagocytes to the infection site

C5a also functions as a **chemoattractant** for phagocytes. Phagocytes will move towards increasing concentrations of C5a and subsequently attach, via their CR1 receptors to the C3b molecules attached to the antigen. This will be discussed in greater detail later in this unit under phagocytosis.

3. Promoting the attachment of antigens to phagocytes (enhanced attachment or opsonization)

C3b and to a lesser extent, **C4b** can function as **opsonins**, that is, they can attach antigens to phagocytes. One portion of the C3b binds to proteins and polysaccharides on microbial surfaces; another portion **attaches to CR1 receptors on phagocytes, B-lymphocytes, and dendritic cells for enhanced phagocytosis (see Fig. 8)**. In actuality, C3b molecule can bind to pretty much any protein or polysaccharide. Human cells, however, produce Factor H that binds to C3b and allows Factor I to inactivate the C3b. On the other hand, substances such as LPS on bacterial cells facilitate the binding of Factor B to C3b and this protects the C3b from inactivation by Factor I. In this way, C3b does not interact with our own cells but is able to interact with microbial cells. C3a and C5a increase the expression of C3b receptors on phagocytes and increase their metabolic activity.

Fig. 8: Illustration of Enhanced Attachment of Bacteria to Phagocytes



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One of the functions of certain antibody molecules known as IgG is to stick antigens such as bacterial proteins and polysaccharides to phagocytes. The "tips" of the antibody, the Fab portion, have a shape that fits epitopes, portions of an antigen with a complementary shape. The "stalk" of the antibody is called the Fc portion and is able to bind to Fc receptors on phagocytes. Also, when body defense pathways known as the complement pathways are activated, one of the beneficial defense proteins made is called C3b. C3b binds by one end to bacterial surface proteins and by the other end to C3b receptors on phagocytes. The IgG and C3b are also known as opsonins and the process of enhanced attachment is also called opsonization.

Flash animation showing the role of C5a in vasodilation, the chemotaxis of phagocytes towards C5a, and their attachment to the opsonin C3b as a result of the complement pathways.
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html5 version of animation for iPad showing the role of C5a in vasodilation, the chemotaxis of phagocytes towards C5a, and their attachment to the opsonin C3b as a result of the complement pathways.
During the complement pathways, complement proteins such as C3a, C3b, C4a, C4b, and C5a are produced. These all play a role in inflammation and phagocytosis. C5a, C3a, and C4a stimulate mast cells to release histamine and other vasoactive agents to promote inflammation and diapedesis. C5a also functions as a chemoattractant for phagocytes. Most C3b and C4b binds to antigens on the microbial surface. The phagocytes are then able to bind to the C3b attached to the surface of the microorganism allowing for opsonization (enhanced attachment).

4. Causing lysis of Gram-negative bacteria, human cells displaying foreign epitopes, and viral envelopes

C5b6789_n, functions as a **Membrane Attack Complex (MAC)**. This helps to **destroy gram-negative bacteria as well as human cells displaying foreign antigens** (virus-infected cells, tumor cells, etc.) by causing their lysis. It can also damage the envelope of enveloped viruses.

Flash animation showing the formation of the Membrane Attack Complex (MAC) and cytolysis during the complement pathways.
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html5 version of animation for iPad showing the formation of the Membrane Attack Complex (MAC) and cytolysis during the complement pathways.
This C5b6789 _n , or membrane attack complex (MAC), puts pores into lipid bilayer membranes of human cells to which antibodies have bound. This results in cell lysis. MAC can also damage the envelope of enveloped viruses and put pores in the outer membrane and cytoplasmic membrane of Gram-negative bacteria causing their lysis.

5. Serving as a second signal for activating naive B-lymphocytes during adaptive immunity

Some C3b is converted to C3d. C3d binds to CR2 receptors on B-lymphocytes. This serves as a second signal for the activation of B-lymphocytes whose B-cell receptors have just interacted with their corresponding antigen.

6. Removing harmful immune complexes from the body

C3b and to a lesser extent, **C4b** help to remove harmful immune complexes from the body. The C3b and C4b attach the immune complexes to CR1 receptors on erythrocytes. The erythrocytes then deliver the complexes to fixed macrophages within the spleen and liver for destruction. Immune complexes can lead to a

harmful Type III hypersensitivity, as will be discussed later in Unit 5 under Hypersensitivities.

Concept Map for the Complement Pathways

TPS Questions

Quiz Group

The Lectin Pathway

The **lectin pathway** is activated by the **interaction of microbial carbohydrates with mannose-binding lectin (MBL)** or ficolins found in the plasma and tissue fluids. (Lectins are carbohydrate-binding proteins.)

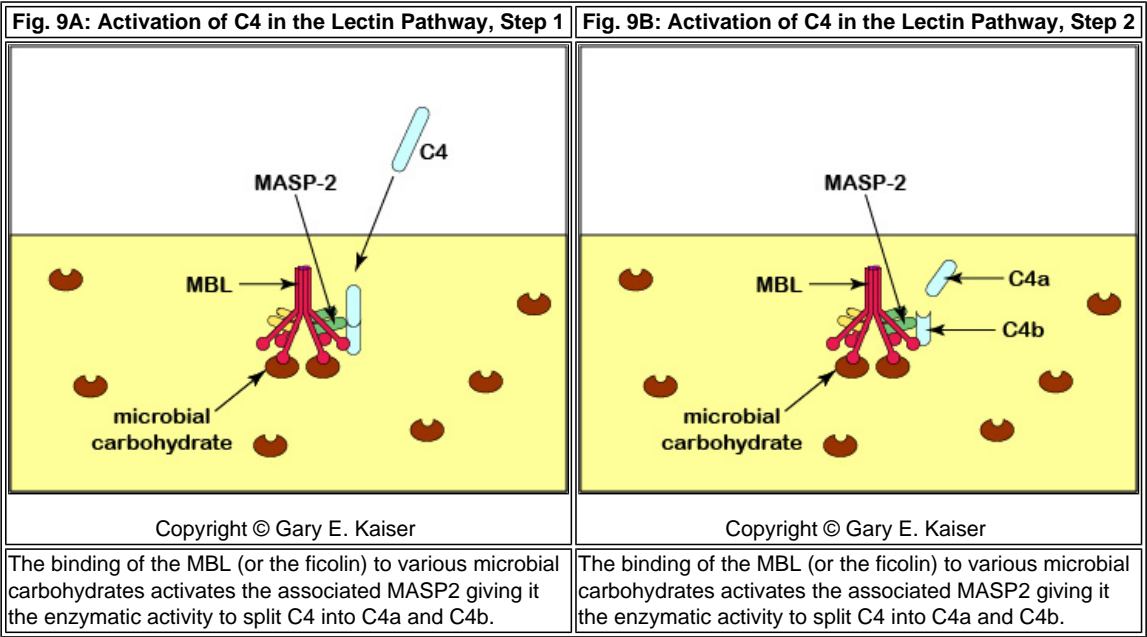
The lectin pathway is mediated by two groups of proteins found in the plasma of the blood and in tissue fluids:

1. **Mannose-binding lectin (MBL)** - also known as mannose-binding protein or MBP. MBL is a soluble pattern-recognition receptor that **binds to various microbial carbohydrates such as those rich in mannose or fucose, and to N-acetylglucosamine (NAG)**. These glycans are common in microbial glycoproteins and glycolipids but rare in those of humans. MBL is synthesized by the liver and released into the bloodstream as part of the acute phase response that will be discussed later in this unit. The MBL is equivalent to C1q in the classical complement pathway.

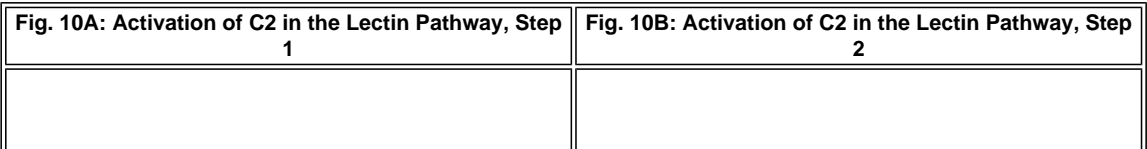
Ficolins are similar in their structure to MBL and bind to microbial carbohydrates such as **N-acetylglucosamine (NAG), lipoteichoic acids, and lipopolysaccharide (LPS)**. Ficolin is also equivalent to C1q in the classical complement pathway.

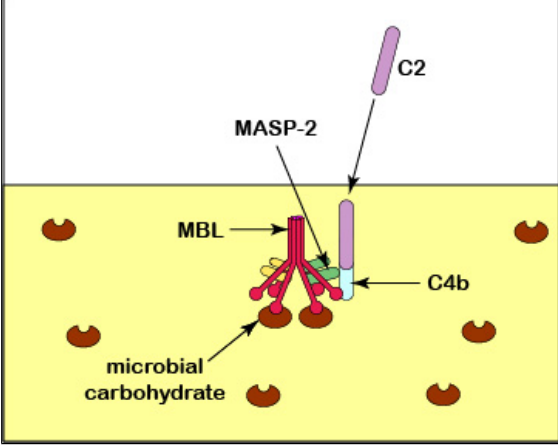
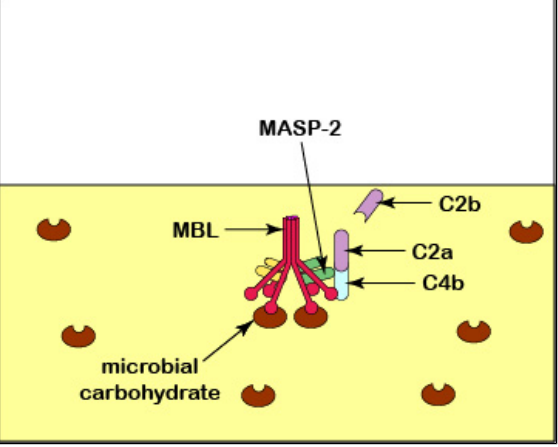
2. Both **mannose-binding lectin (MBL)** and **ficolin** form complexes with MBL-associated serine proteases called **MASP1 and MASP2**, which are equivalent to C1r and C1s of the classical pathway.

a. The **binding of the MBL (or the ficolin) to the microbial carbohydrate activates the associated MASP2** giving it the enzymatic activity to **split C4 into C4a and C4b** (see Fig. 9A and Fig. 9B).



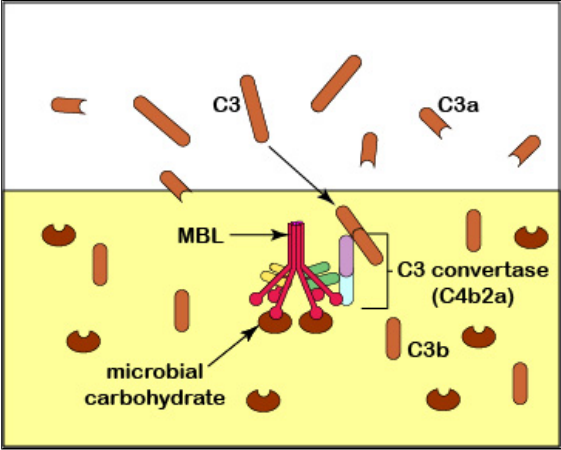
b. **C2 then binds to C4b and is cleaved by MASP2 into C2a and C2b** (see Fig. 10A and Fig. 10B).



	
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C2 binds to C4a and is split into C2a and C2b. The C4bC2a then form a C3 convertase that can now cleave hundreds of molecules of C3 into C3a and C3b.	C2 binds to C4a and is split into C2a and C2b. The C4bC2a then form a C3 convertase that can now cleave hundreds of molecules of C3 into C3a and C3b.

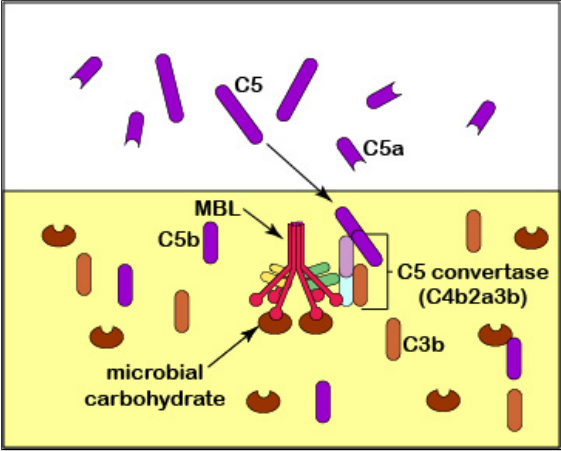
Flash animation showing activation of the lectin pathway
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing activation of the lectin pathway
The lectin pathway is activated by the interaction of various microbial carbohydrates such as mannose, fucose, N-acetylglucosamine (NAG), lipoteichoic acids, and lipopolysaccharide (LPS) with mannose-binding lectin (MBL) or ficolins which forms complexes with two proteases called MASP1 and MASP2 in the plasma. (Lectins are carbohydrate-binding proteins.) The binding of the MBL (or the ficolin) to the microbial carbohydrate activates the associated MASP2 giving it the enzymatic activity to split C4 into C4a and C4b, as well as C2 into C2a and C2b.

c. **C4b and C2a combine to form C4b2a, the C3 convertase.** C3 convertase can now **cleave hundreds of molecules of C3 into C3a and C3b** (see Fig. 11).

Fig. 11: Activation of C3 in the Lectin Pathway

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C4b and C2a combine to form C4b2a, the C3 convertase. C3 convertase can now cleave hundreds of molecules of C3 into C3a and C3b.

d. Some molecules of C3b bind to C4b2a, the C3 convertase, to form **C4b2a3b, a C5 convertase** that **cleaves C5 into C5a and C5b** (see Fig. 12).

Fig. 12: Activation of C5 in the Lectin Pathway

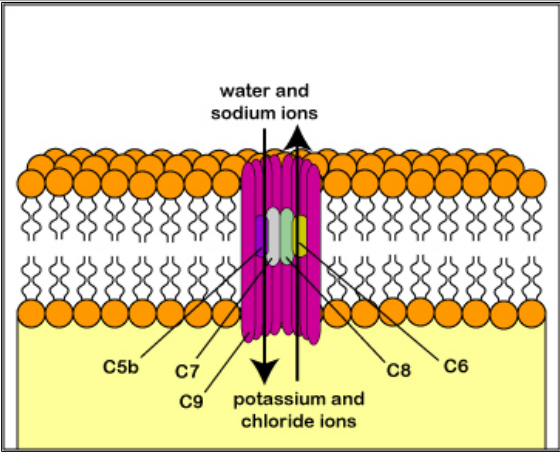
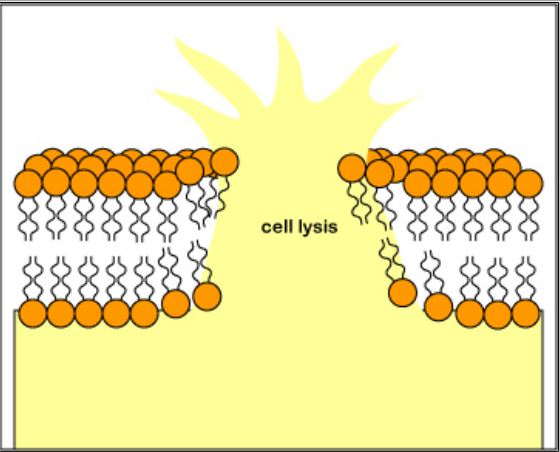


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Some molecules of C3b bind to C4b2a, the C3 convertase, to form C4b2a3b, a C5 convertase that cleaves C5 into C5a and C5b.

Flash animation showing formation of C3 convertase and C5 convertase during the lectin pathway
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html5 version of animation for iPad showing formation of C3 convertase and C5 convertase during the lectin pathway.
The MBL-associated MASP2 is able to cleave C4 into C4a and C4b. The C4b binds to adjacent proteins and carbohydrates on the surface of the antigen. C2 then binds to the C4b and MASP2 cleaves C2 into C2a and C2b. The C4b2a functions as a C3 convertase that can subsequently cleave hundreds of molecules of C3 into C3a and C3b. C3b attaches antigens to phagocytes for opsonization (enhanced attachment) while C3a can promote inflammatory responses that enable body defense cells and defense chemicals to leave the blood and enter the tissues.
Some of the C3b combines with the C4b and the C2a. C4b2a3b functions as a C5 convertase that cleaves molecules of C5 into C5a and C5b. C5a is the most potent complement protein triggering inflammation. C5b becomes part of the Membrane Attack Complex (MAC).

e. C5b binds to the surface of the target cell and subsequently binds C6, C7, C8, and a number of monomers of C9 to form **C5b6789_n**, the **Membrane Attack Complex (MAC)** (see Fig. 6 and Fig. 7).

Fig. 6: Illustration of Formation of the Membrane Attack Complex (MAC) during the Complement Pathways	Fig. 7: Illustration of Membrane Attack Complex (MAC)-Induced Lysis during the Complement Pathways
 <p>Copyright © Gary E. Kaiser</p> <p>Multiple molecules of C9 combine with C5b, C6, C7, and C8 to form the Membrane Attack Complex (MAC). C5b6789_n functions as a Membrane Attack Complex (MAC). This helps to destroy Gram-negative bacteria as well as human cells displaying foreign antigens (virus-infected cells, tumor cells, etc.) by causing their lysis. It can also damage the envelope of enveloped viruses.</p>	 <p>Copyright © Gary E. Kaiser</p> <p>Multiple molecules of C9 combine with C5b, C6, C7, and C8 to form the Membrane Attack Complex (MAC). C5b6789_n functions as a Membrane Attack Complex (MAC). This helps to destroy Gram-negative bacteria as well as human cells displaying foreign antigens (virus-infected cells, tumor cells, etc.) by causing their lysis. It can also damage the envelope of enveloped viruses.</p>

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This C5b6789 _n , or membrane attack complex (MAC), puts pores into lipid bilayer membranes of human cells to which antibodies have bound. This results in cell lysis. MAC can also damage the envelope of enveloped viruses and put pores in the outer membrane and cytoplasmic membrane of Gram-negative bacteria causing their lysis.

The beneficial results of the activated complement proteins are the same as in the classical complement pathway above. The complement proteins:

- 1. **Trigger inflammation:** C5a>C3a>c4a;
- 2. **Chemotactically attract phagocytes to the infection site:** C5a;
- 3. **Promote the attachment of antigens to phagocytes via enhanced attachment or opsonization:** C3b>C4b;
- 4. **Cause lysis of Gram-negative bacteria and human cells displaying foreign epitopes:** MAC;
- 5. **Serve as a second signal for the activation of naive B-lymphocytes:** C3d; and
- 6. **Remove harmful immune complexes from the body:** C3b>C4b.

Flash animation showing the role of C5a in vasodilation, the chemotaxis of phagocytes towards C5a, and their attachment to the opsonin C3b as a result of the complement pathways.
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During the complement pathways, complement proteins such as C3a, C3b, C4a, C4b, and C5a are produced. These all play a role in inflammation and phagocytosis. C5a, C3a, and C4a stimulate mast cells to release histamine and other vasoactive agents to promote inflammation and diapedesis. C5a also functions as a chemoattractant for phagocytes. Most C3b and C4b binds to antigens on the microbial surface. The phagocytes are then able to bind to the C3b attached to the surface of the microorganism allowing for opsonization (enhanced attachment).

Concept Map for the Complement Pathways

The Alternative Complement Pathway

The alternative complement pathway is **mediated by C3b**, produced either by the classical or lectin pathways or from C3 hydrolysis by water. (Water can hydrolyze C3 and form C3i, a molecule that functions in a manner similar to C3b.)

- 1. Activation of the alternative complement pathway begins when **C3b (or C3i) binds to the cell wall and other surface components of microbes**. C3b can also bind to IgG antibodies. Alternative pathway protein **Factor B** then combines with the cell-bound C3b to form **C3bB**. **Factor D** then splits the bound Factor B into Bb and Ba, forming **C3bBb**. A serum protein called **properdin** then binds to the Bb to form **C3bBbP that functions as a C3 convertase (see Fig. 13)** capable of enzymatically splitting hundreds of molecules of C3 into C3a and C3b. The alternative complement pathway is now activated.

Fig. 13: Activation of the Alternative Complement Pathway and Formation of C3 Convertase



2. Some of the C3b subsequently binds to some of the C3bBb to form **C3bBb3b, a C5 convertase** capable of splitting molecules of C5 into C5a and C5b (see **Fig. 14**). From here, the alternative complement pathway is identical to the other complement pathways.

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Some C3b binds to C3bBb to form C3bBb3b. C3bBb3b functions as a C5 convertase that can enzymatically split hundreds of molecules of C5 into C5a and C5b.

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Activation of the alternative complement pathway begins when C3b (or C3i) binds to the cell wall and other surface components of microbes. Alternative pathway protein Factor B then combines with the cell-bound C3b to form C3bB. Factor D then splits the bound Factor B into Bb and Ba, forming C3bBb. A serum protein called properdin then binds to the Bb to form C3bBbP that functions as a C3 convertase capable of enzymatically splitting hundreds of molecules of C3 into C3a and C3b. Some C3b binds to C3bBb to form C3bBb3b. C3bBb3b functions as a C5 convertase that can enzymatically split hundreds of molecules of C5 into C5a and C5b.

1. **Trigger inflammation:** C5a>C3a>c4a;
2. **Chemotactically attract phagocytes to the infection site:** C5a;

- 3. **Promote the attachment of antigens to phagocytes via enhanced attachment or opsonization:** C3b>C4b;
- 4. **Cause lysis of Gram-negative bacteria, human cells displaying foreign epitopes, and viral envelopes:** MAC; and
- 5. **Serve as a second signal for the activation of naive B-lymphocytes:** C3d;
- 6. **Remove harmful immune complexes from the body:** C3b>C4b.

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Concept Map for the Complement Pathways

Keep in mind that in Unit 3 under "Virulence Factors that Promote Bacterial Colonization of the Host" we learned several mechanisms that various bacteria use to resist the body's complement pathways. By resisting these immediate innate immune defenses, some bacteria have a better chance of colonizing their host.

Self Quiz for the Complement Pathways



[Return to Unit 5 and 6 Table of Contents](#)

[Back to Softchalk Lessons Table of Contents](#)

Immediate Innate Immunity: Anatomical Barriers, Mechanical Removal, and Bacterial Antagonism by Normal Microbiota



1. Anatomical barriers such as the skin, the mucous membranes, and bony encasements are tough, intact barriers that prevent the entry and colonization of many microbes.
2. Mechanical removal is the process of physically flushing microbes from the body. Examples include mucus and cilia, coughing and sneezing, vomiting and diarrhea, and the flushing action of bodily fluids.
3. The normal microbiota keeps potentially harmful opportunistic pathogens in check and also inhibits the colonization of pathogens by producing metabolic products that inhibit the growth of many pathogens, adhering to target host cells so as to cover them and prevent pathogens from colonizing, depleting nutrients essential for the growth of pathogens, and non-specifically stimulating the immune system.
4. Destruction of normal bacterial microbiota by the use of broad spectrum antibiotics may result in superinfections or overgrowth by antibiotic-resistant opportunistic microbiota such as *Candida* and *Clostridium difficile*.

1. Identify the functions of the anatomical barriers and physiological barriers that make up innate immunity.
2. Describe how each of the anatomic and physiological barriers prevent invasion/ infection with microbes.
3. Describe the role of the body's normal flora in preventing invasion/infection with opportunistic and pathogenic microbes.

Detailed Learning Objectives

- 1. Describe what is meant by anatomical barriers to infection.
- 2*. List 4 ways in which the body can physically remove microorganisms or their products.
- 3. Briefly describe how intraepithelial T-lymphocytes and B-1 cells play a role in innate immunity.
- 4**. Describe how bacterial antagonism by normal microbiota acts as a non-specific body defense mechanism and name 2 opportunistic microbes that may cause superinfection upon destruction of the normal microbiota.
- 5. Briefly describe the process involved in the development of antibiotic-associated colitis.

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Questions

Immediate Innate Immunity: Anatomical Barriers, Mechanical Removal, and Bacterial Antagonism by Normal Microbiota

Immediate innate immunity begins 0-4 hours after exposure to an infectious agent and involves the action of soluble preformed antimicrobial molecules that circulate in the blood, our found in extracellular tissue fluids, and are secreted by epithelial cells. These include:

- antimicrobial enzymes and peptides, and
- complement system proteins.

These preformed antimicrobial molecules are designed to immediately begin to remove infectious agents as soon as they enter the body.

In addition to preformed antimicrobial molecules, the following also play a role in immediate innate immunity:

- anatomical barriers to infection
- mechanical removal of microbes
- bacterial antagonism by the body's normal microbiota

In this section we will look at how anatomical barriers, mechanical removal, and bacterial antagonism by normal body microbiota function to prevent infection.

Anatomical Barriers

Anatomical barriers are tough, intact barriers that prevent the entry and colonization of many microbes. Examples include the skin, the mucous membranes, and bony encasements.

1. The skin

The skin, consisting of the epidermis and the dermis, is **dry, acidic, and has a temperature lower than 37 degrees Celsius** (body temperature). These conditions are not favorable to bacterial growth. **Resident normal microbiota** of the skin also inhibits potentially harmful microbes. In addition, the **dead, keratinized cells that make up the surface of the skin are continuously being sloughed off** so that microbes that do colonize these cells are constantly being removed. Hair follicles and sweat glands produce **lysozyme and toxic lipids** that can kill bacteria. Epithelial cells also produce defensins and cathelicidins to kill microbes. Beneath the epidermis of the skin are Langerhans' cells - immature dendritic cells - that phagocytose and kill microbes, carry them to nearby lymph nodes, and present antigens of these microbes to T-lymphocytes to begin adaptive immune responses against them. Finally, intraepithelial T-lymphocytes and B-1 lymphocytes are associated with the epidermis and the mucosal epithelium. These cells recognize microbes common to the epidermis and mucous membranes and start immediate adaptive immune responses against these commonly encountered microbes.

2. The mucous membranes

Mucous membranes line body cavities that open to the exterior, such as the respiratory tract, the gastrointestinal tract, and the genitourinary tract. Mucous membranes are composed of an epithelial layer that secretes mucus, and a connective tissue layer. The **mucus is a physical barrier that traps microbes**. Mucus also contains **lysozyme** to degrade bacterial peptidoglycan, an antibody called **secretory IgA** that prevents microbes from attaching to mucosal cells and traps them in the mucous, **lactoferrin** to bind iron and keep it from from being used by microbes, and **lactoperoxidase** to generate toxic superoxide radicals that kill microbes. **Resident normal microbiota** of the mucosa also inhibits potentially harmful microbes. In addition, the mucous membrane, like the skin, is

constantly sloughing cells to remove microbes that have attached to the mucous membranes. Beneath the mucosal membrane is **mucosa-associated lymphoid tissue (MALT)** that contains Langerhans' cells - immature dendritic cells - that phagocytose and kill microbes, carry them to nearby lymph nodes, and present antigens of these microbes to T-lymphocytes to begin adaptive immune responses against them. Intraepithelial T-lymphocytes and B-1 lymphocytes are associated with the epidermis and the mucosal epithelium. These cells recognize microbes common to the epidermis and mucous membranes and start immediate adaptive immune responses against these commonly encountered microbes.

3. **Bony encasements**

Bony encasements, such as the skull and the thoracic cage, protect vital organs from injury and entry of microbes.

Mechanical Removal

Mechanical removal is the process of physically flushing microbes from the body. Methods include:

1. **Mucus and cilia**

Mucus traps microorganisms and prevents them from reaching and colonizing the mucosal epithelium. Mucus also contains **lysozyme** to degrade bacterial peptidoglycan, an antibody called **secretory IgA** that prevents microbes from attaching to mucosal cells and traps them in the mucus, **lactoferrin** to bind iron and keep it from being used by microbes, and **lactoperoxidase** to generate toxic superoxide radicals that kill microbes. **Cilia** on the surface of the epithelial cells **propel mucus and trapped microbes upwards towards the throat where it is swallowed** and the microbes are killed in the stomach. This is sometimes called the tracheal toilet.

2. **The cough and sneeze reflex**

Coughing and sneezing removes mucus and trapped microbes.

3. **Vomiting and diarrhea**

These processes remove pathogens and toxins in the gastrointestinal tract.

4. **The physical flushing action of body fluids**

Fluids such as urine, tears, saliva, perspiration, and blood from injured blood vessels also flush microbes from the body.

Bacterial Antagonism by Normal Microbiota

Bacterial antagonism is the process by which **the body's normal microbiota inhibit the growth or colonization of opportunistic pathogens and pathogens**. Approximately 100 trillion bacteria and other microorganisms reside in or on the human body. The normal body microbiota keeps potentially harmful opportunistic pathogens in check and also inhibits the colonization of pathogens by:

- 1. Producing **metabolic products** (fatty acids, bacteriocins, etc.) that **inhibit the growth of many pathogens**;
- 2. **Adhering to target host cells** so as to cover them and **preventing pathogens from colonizing**;
- 3. **Depleting nutrients** essential for the growth of pathogens; and
- 4. **Non-specifically stimulating the immune system**.

Destruction of normal bacterial microbiota by the use of broad spectrum antibiotics may result in **superinfections** or overgrowth by antibiotic-resistant opportunistic microbiota. For example, the yeast *Candida*, that causes infections such as vaginitis and thrush, and the bacterium *Clostridium difficile*, that causes potentially severe antibiotic-associated colitis, are opportunistic microorganisms normally held in check by the normal microbiota.

In the case of *Candida* infections, the *Candida* resists the antibacterial antibiotics because being a yeast, it is eukaryotic, not prokaryotic like the bacteria. Once the bacteria are eliminated by the antibiotics, the *Candida* has no competition and can overgrow the area.

Clostridium difficile is an opportunistic Gram-positive, endospore-producing bacillus transmitted by the fecal-oral route that causes severe antibiotic-associated colitis. *C. difficile* is a common healthcare-associated infection (HAIs) and is the most frequent cause of health-care-associated diarrhea. *C. difficile* infection often recurs and can progress to sepsis and death. *C. difficile* infection often recurs and can progress to sepsis and death. CDC has estimated that there are about 500,000 *C. difficile* infections (CDI) in health-care associated patients each year and is linked to 15,000 American deaths each year.

Antibiotic-associated colitis is especially common in older adults. It is thought that *C. difficile* survives the exposure to the antibiotic by sporulation. After the antibiotic is no longer in the body, the endospores germinate and *C. difficile* overgrows the intestinal tract and secretes toxin A and toxin B that have a cytotoxic effect on the epithelial cells of the colon. *C.difficile* has become increasingly resistant to antibiotics in recent years making treatment often difficult. There has been a great deal of success in treating the infection with fecal transplants, still primarily an experimental procedure. Polymerase chain reaction (PCRs) assays, which test for the bacterial gene encoding toxin B, are highly sensitive and specific for the presence of a toxin-producing *Clostridium difficile* organism. The most successful technique in restricting *C. difficile* infections has been the restriction of the use of antimicrobial agents.

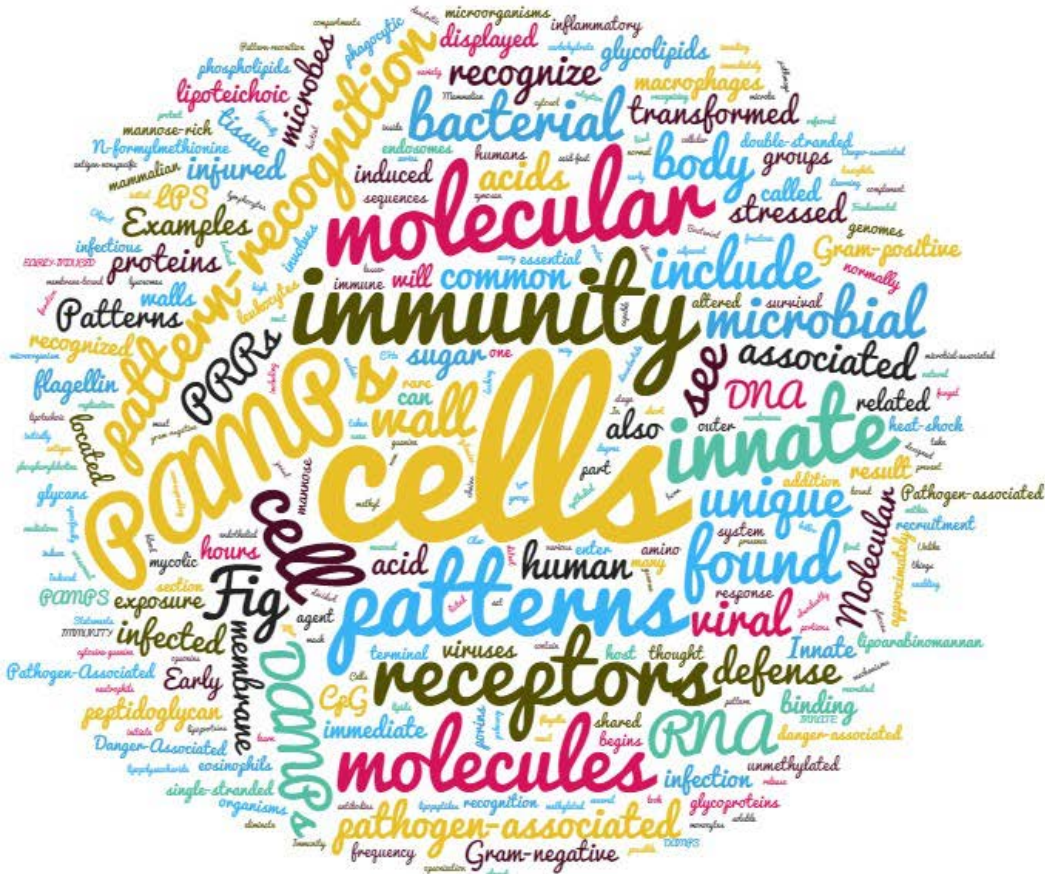
TPS Questions

Quiz Group

Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

Early Induced Innate Immunity: Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)



1. Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPs binding to pattern-recognition receptors or PRRs.
2. Pathogen-associated molecular patterns or PAMPs are molecules shared by groups of related microbes that are essential for the survival of those organisms and are not found associated with mammalian cells. Examples include LPS, porins, peptidoglycan, lipoteichoic acids, mannose-rich glycans, flagellin, bacterial and viral genomes, mycolic acid, and lipoarabinomannan.
3. Danger-associated molecular patterns or DAMPs are unique molecules displayed on stressed, injured, infected, or transformed human cells also be recognized as a part of innate immunity. Examples include heat-shock proteins and altered membrane phospholipids.
4. PAMPs and DAMPs bind to pattern-recognition receptors or PRRs associated with body cells to induce innate immunity.

1. Explain the role of PAMPs, DAMPs, and PRRs in innate immunity.

Detailed Learning Objectives

1. State how long it takes for early induced innate immunity to become activated and what it involves.
- 2.* State what is meant by pathogen-associated molecular patterns (PAMPs), and the role PAMPs play in inducing innate immunity.
3. Name at least 5 PAMPs associated with bacteria.
4. Name at least 2 PAMPs associated with viruses.
5. Define DAMPs and give two examples.

(*) or (**) = possible discussion question

TPS Questions

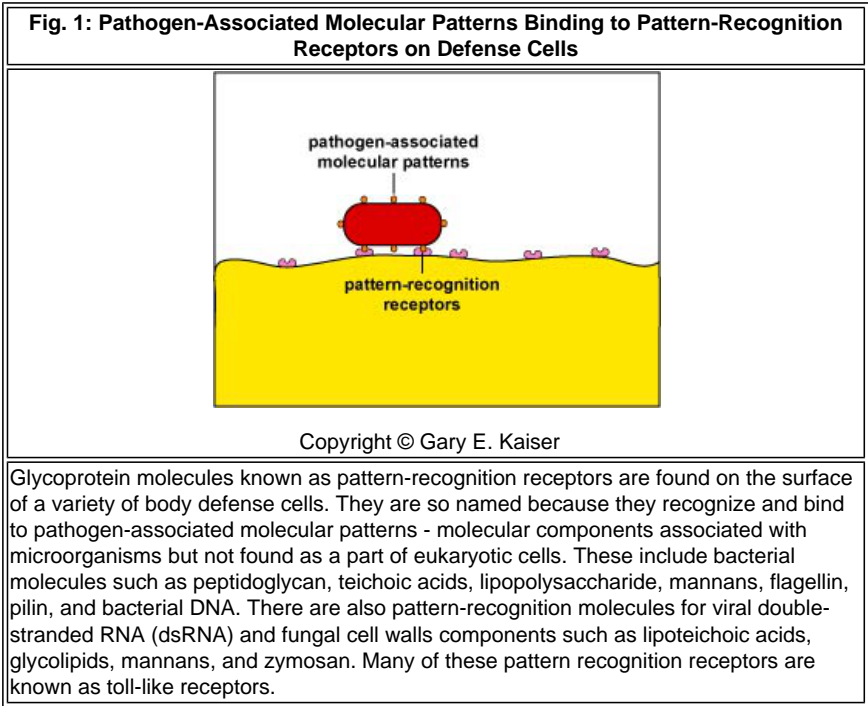
Early Induced Innate Immunity: Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)

Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPs binding to pattern-recognition receptors or PRRs. These recruited defense cells include:

- phagocytic cells: leukocytes such as neutrophils, eosinophils, and monocytes; tissue phagocytic cells in the tissue such as macrophages;
- cells that release inflammatory mediators: inflammatory cells in the tissue such as macrophages and mast cells; leukocytes such as basophils and eosinophils; and
- natural killer cells (NK cells).

Unlike adaptive immunity, innate immunity does not recognize every possible antigen. Instead, it is **designed to recognize molecules shared by groups of related microbes that are essential for the survival of those organisms** and are not found associated with mammalian cells. These unique microbial molecules are called **pathogen-associated molecular patterns** or PAMPs and include LPS from the Gram-negative cell wall, peptidoglycan and lipoteichoic acids from the Gram-positive cell wall, the sugar mannose (a terminal sugar common in microbial glycolipids and glycoproteins but rare in those of humans), bacterial and viral unmethylated CpG DNA, bacterial flagellin, the amino acid *N*-formylmethionine found in bacterial proteins, double-stranded and single-stranded RNA from viruses, and glucans from fungal cell walls. In addition, **unique molecules displayed on stressed, injured, infected, or transformed human cells** also be recognized as a part of innate immunity. These are often referred to as **danger-associated molecular patterns or DAMPs**.

Most body defense cells have pattern-recognition receptors or PRRs for these common PAMPs (see Fig. 1) and so there is an **immediate response** against the invading microorganism. Pathogen-associated molecular patterns can also be recognized by a series of soluble pattern-recognition receptors in the blood that function as opsonins and initiate the complement pathways. In all, the innate immune system is thought to recognize approximately 10^3 of these microbial molecular patterns.



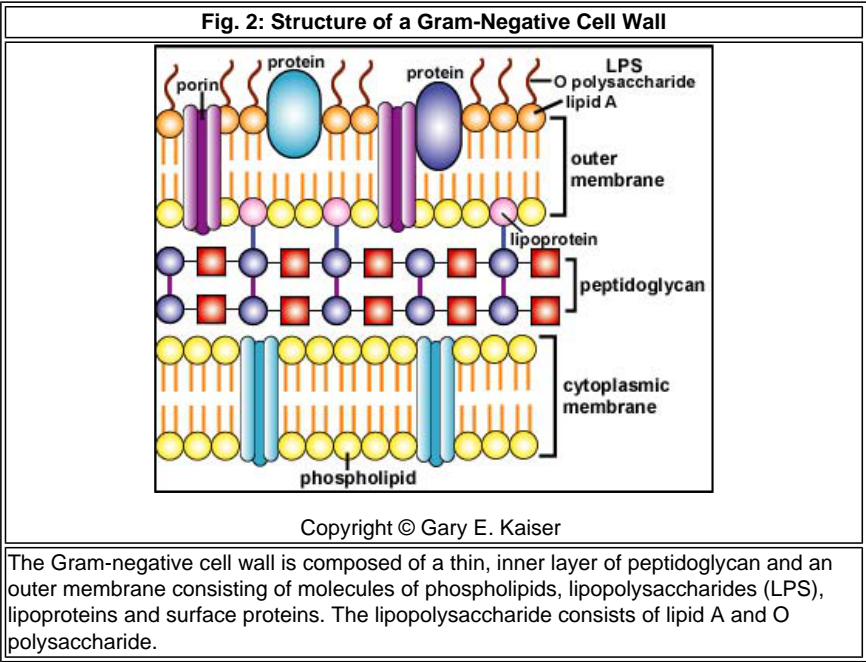
We will now take a closer look at pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs).

Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)

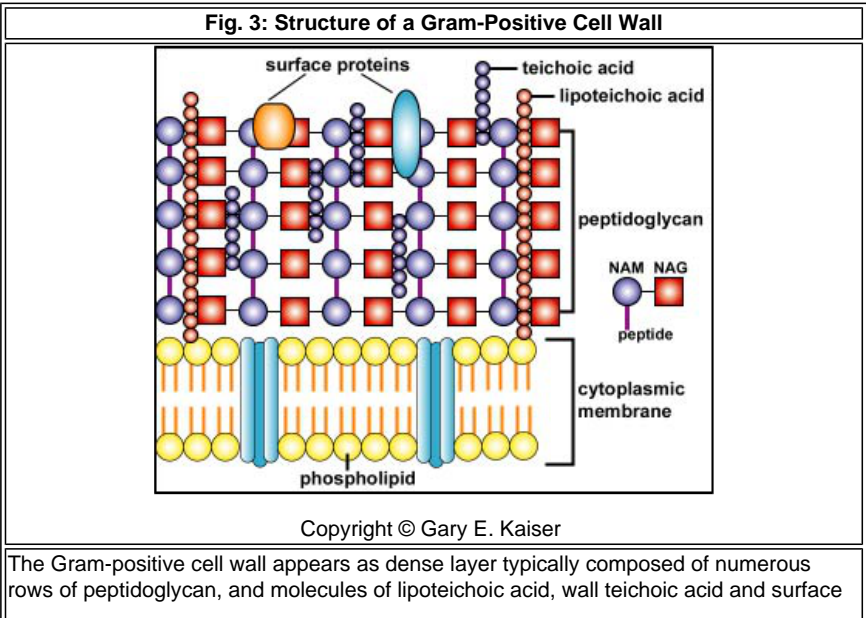
In order to protect against infection, one of the first things the body must do is detect the presence of microorganisms. The body initially does this by **recognizing molecules unique to groups of related microorganisms and are not associated with human cells**. These unique microbial molecules are called **pathogen-associated molecular patterns or PAMPs**. In addition, **unique molecules displayed on stressed, injured, infected, or transformed human cells** also be recognized as a part of innate immunity. These are often referred to as **danger-associated molecular patterns or DAMPs**. In all, the innate immune system is thought to recognize approximately 10^3 molecular patterns.

Examples of microbial-associated PAMPs include:

- a. **lipopolysaccharide (LPS)** from the outer membrane of the Gram-negative cell wall (see Fig. 2);
- b. bacterial lipoproteins and lipopeptides (see Fig. 2);
- c. porins in the outer membrane of the Gram-negative cell wall (see Fig. 2);

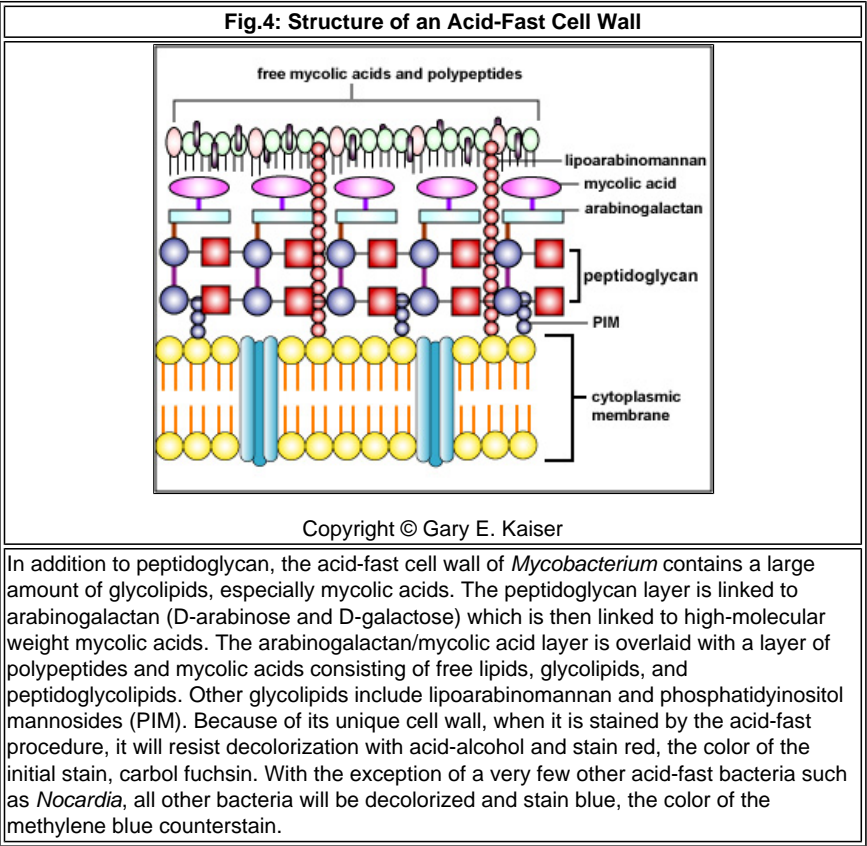


- d. **peptidoglycan** found abundantly in the Gram-positive cell wall and to a lesser degree in the gram-negative cell wall (see Fig. 3);
- e. **lipoteichoic acids** found in the Gram-positive cell wall (see Fig. 3);

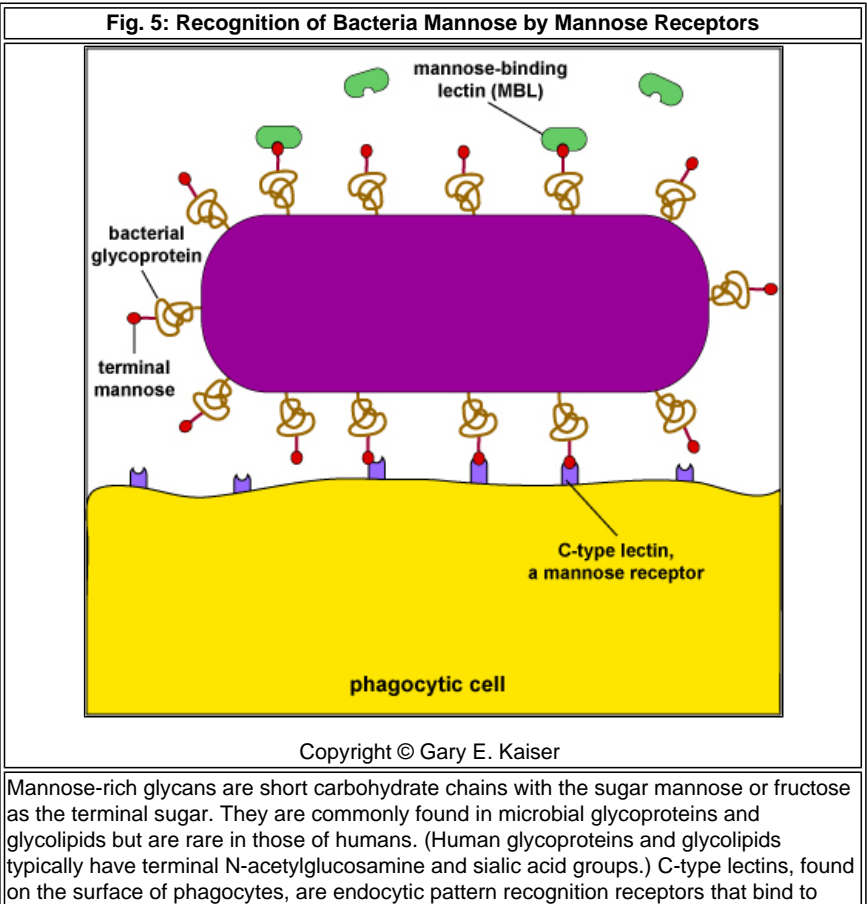


proteins.

f. **lipoarabinomannan and mycolic acids** found in acid-fast cell walls (see Fig. 4)

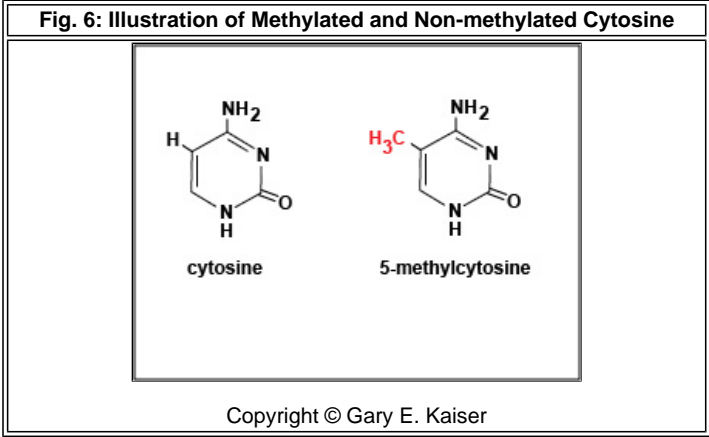


g. **mannose-rich glycans** (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar). These are common in microbial glycoproteins and glycolipids but rare in those of humans (see Fig. 5).



mannose-rich glycans in order to attach microbes to phagocytes. Mannose-binding lectin (MBL), also known as mannan-binding protein, is a soluble pattern recognition receptor in plasma and tissue fluid that binds to mannose-rich glycans on microbes in order to activate the lectin complement pathway.

- h. **flagellin** found in bacterial flagella;
- i. **bacterial and viral nucleic acid**. Bacterial and viral genomes contain a high frequency of unmethylated cytosine-guanine dinucleotide or CpG sequences (a cytosine lacking a methyl or CH₃ group (**see Fig. 6**) and located adjacent to a guanine). Mammalian DNA has a low frequency of CpG sequences and most are methylated which may mask recognition by pattern-recognition receptors. Also, human DNA and RNA does not normally enter cellular endosomes where the pattern-recognition receptors for microbial DNA and RNA are located;



- j. **N-formylmethionine**, an amino acid common to bacterial proteins;
- k. **double-stranded viral RNA** unique to many viruses in some stage of their replication;
- l. **single-stranded viral RNA** from many` viruses having an RNA genome;
- m. **lipoteichoic acids, glycolipids, and zymosan** from yeast cell walls; and
- n. **phosphorylcholine and other lipids** common to microbial membranes.

Examples of DAMPs associated with stressed, injured, infected, or transformed host cells and not found on normal cells include:

- a. heat-shock proteins;
- b. **altered membrane phospholipids**; and
- c. **molecules normally located inside phagosomes and lysosomes that enter the cytosol only when these membrane-bound compartments are damaged as a result of infection**, including antibodies bound to microbes from opsonization.
- d. **molecules normally found within cells, such as ATP, DNA, and RNA, that spill out of damaged cells**.

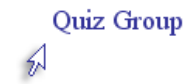
To recognize PAMPs such as those listed above, various body cells have a variety of corresponding receptors called **pattern-recognition receptors or PRRs** capable of binding specifically to conserved portions of these molecules. Cells that typically have pattern recognition receptors include macrophages, dendritic cells, endothelial cells, mucosal epithelial cells, and lymphocytes.

For more information: Preview of pattern-recognition receptors (PRRs)

Concept Map for PAMPs and PRRs

TPS Questions

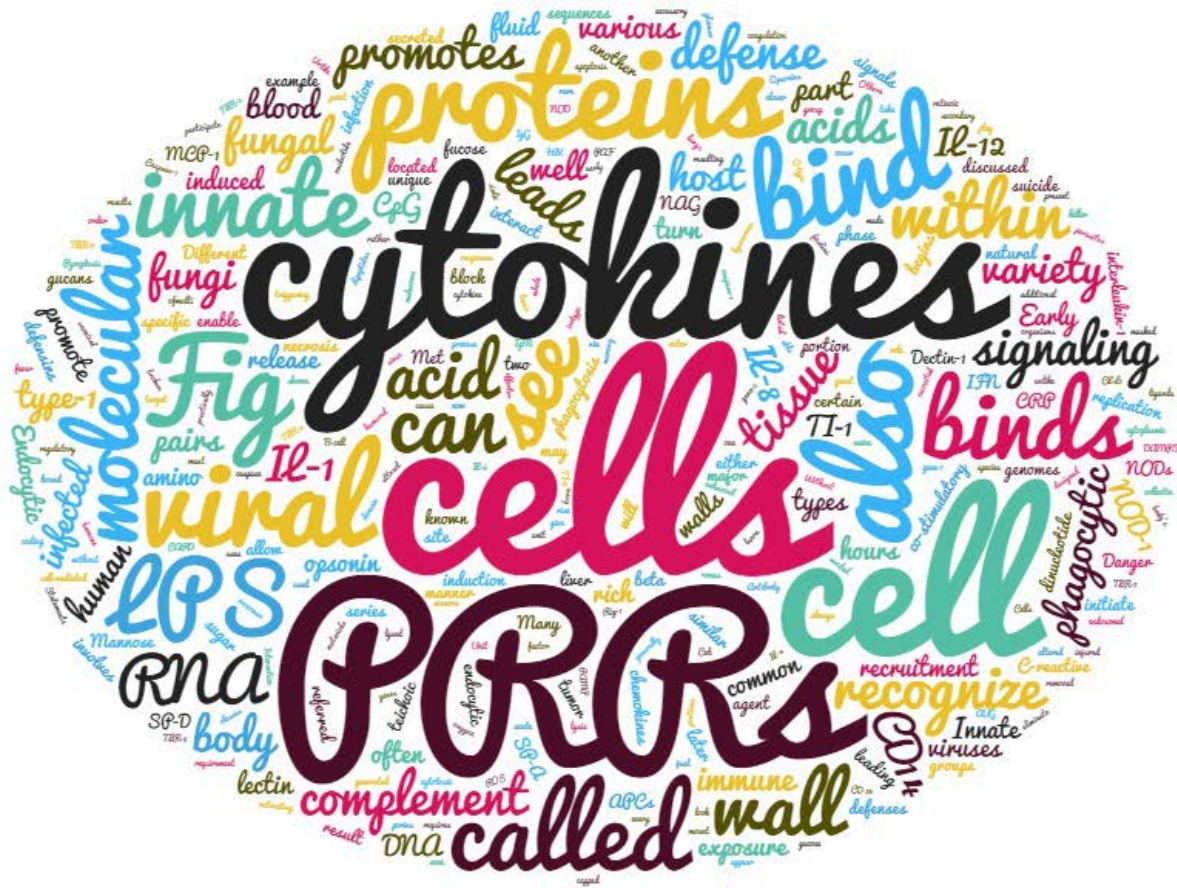
Self Quiz for Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)



[Return to Unit 5 and 6 Table of Contents](#)

[Back to Softchalk Lessons Table of Contents](#)

Early Induced Innate Immunity: Pattern-Recognition Receptors (PRRs) and Danger-Recognition Receptors (DRRs)



1. Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPs binding to pattern-recognition receptors or PRRs and danger-associated molecular patterns or DAMPs binding to danger-recognition receptors or DRRs.
2. Endocytic pattern-recognition receptors are found on the surface of phagocytes and promote the attachment of microorganisms to phagocytes leading to their subsequent engulfment and destruction. They include mannose receptors, scavenger receptors, and opsonin receptors.
3. Binding of microbial PAMPs to signaling PRRs promotes the production of inflammatory cytokines, antiviral cytokines called type-1 interferons (IFN), chemotactic factors, and antimicrobial peptides. They include toll-like receptors (TLRs) and NODs.
4. PRRs found on the surface of the body's cells typically bind to surface PAMPs on microbes and stimulate the production of inflammatory cytokines.
5. PRRs found within cellular phagolysosomes (endosomes) typically detect nucleic acid PAMPs released during the phagocytic destruction of viruses and stimulate the production of antiviral cytokines called type-1 interferons.
6. PRRs and DRRs found within the cytoplasm of host cells typically trigger the formation of multi-protein complexes called inflammasomes which, in turn, triggers the formation of inflammatory cytokines and can also lead to an inflammatory response-induced cell suicide called pyroptosis.
7. PRRs circulating in the blood and tissue fluid activate the complement pathways and may function as opsonins.

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- 1. Explain the role of PAMPs, DAMPs, and PRRs in innate immunity.

Detailed Learning Objectives

- 1. State the function of the following as they relate to innate immunity.
 - a*. pattern recognition receptors (PRRs)
 - b*. endocytic pattern recognition receptors
 - c*. signaling pattern recognition receptors
 - d*. danger-associated molecular patterns
 - e*. danger recognition receptors
 - f. inflammasome
 - g. pyroptosis
- 2. Name 2 endocytic PRRs.
- 3. Name 2 signaling PRRs found on host cell surfaces.
- 4. Name 2 signaling PRRs found in the endosomes of phagocytic cells.
- 5. Name 2 signaling PRRs found on the host cell cytoplasm.
- 6*. Briefly describe the major difference between the effect of the cytokines produced in response to PAMPs that bind to cell surface signaling PRRs and endosomal PRRs.

(*) = Common theme throughout the course

TPS Questions

Early Induced Innate Immunity: Pattern-Recognition Receptors (PRRs) and Danger-Recognition Receptors (DRRs)

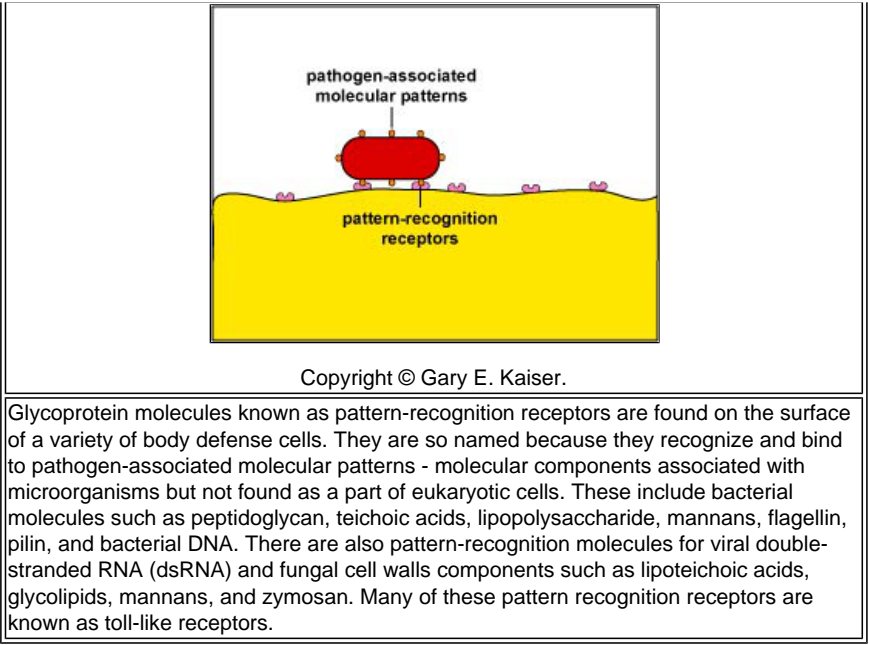
Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPS binding to pattern-recognition receptors or PRRs. These recruited defense cells include:

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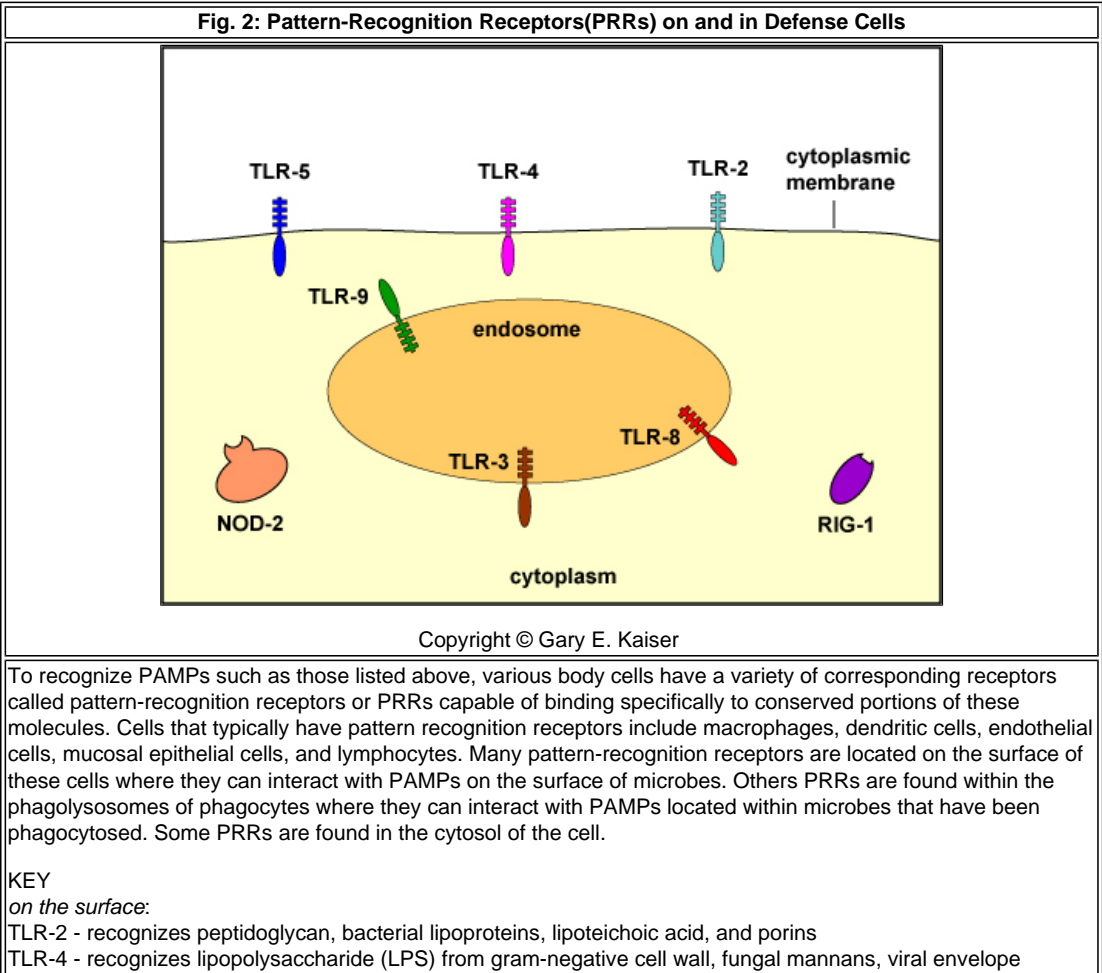
Most body defense cells have pattern-recognition receptors or PRRs for these common PAMPS (**see Fig. 1**) and so there is an **immediate response** against the invading microorganism. Pathogen-associated molecular patterns can also be recognized by a series of soluble pattern-recognition receptors in the blood that function as opsonins and initiate the complement pathways. In all, the innate immune system is thought to recognize approximately 10^3 of these microbial molecular patterns.

Fig. 1: Pathogen-Associated Molecular Patterns Binding to Pattern-Recognition Receptors on Defense Cells



Pattern-Recognition Receptors (PRRs) and Danger-Recognition Receptors (DRRs)

In order to recognize PAMPs, various body cells have a variety of corresponding receptors called **pattern-recognition receptors or PRRs** (see Fig. 2) capable of binding specifically to conserved portions of these molecules. Cells that typically have pattern recognition receptors include macrophages, dendritic cells, endothelial cells, mucosal epithelial cells, and lymphocytes.



proteins, parasitic phospholipids, heat-shock proteins
TLR-5 - recognizes bacterial flagellin

within endosomes (phagolysosomes):
TLR-3 - recognizes viral double-stranded DNA
TLR-8 - recognizes viral single-stranded RNA
TLR-9 - recognizes viral and bacterial unmethylated CpG sequences

in the cytoplasm:
NOD-2 - recognizes muramyl dipeptide from bacterial peptidoglycan
RIG-1 - recognizes viral RNA

For more information: Review of pathogen-associated molecular patterns (PAMPs)

Many pattern-recognition receptors are located on the surface of these cells where they can interact with PAMPs on the surface of microbes. Others PRRs are found within the phagolysosomes of phagocytes where they can interact with PAMPs located within microbes that have been phagocytosed. Some PRRs are found in the cytosol of the cell.

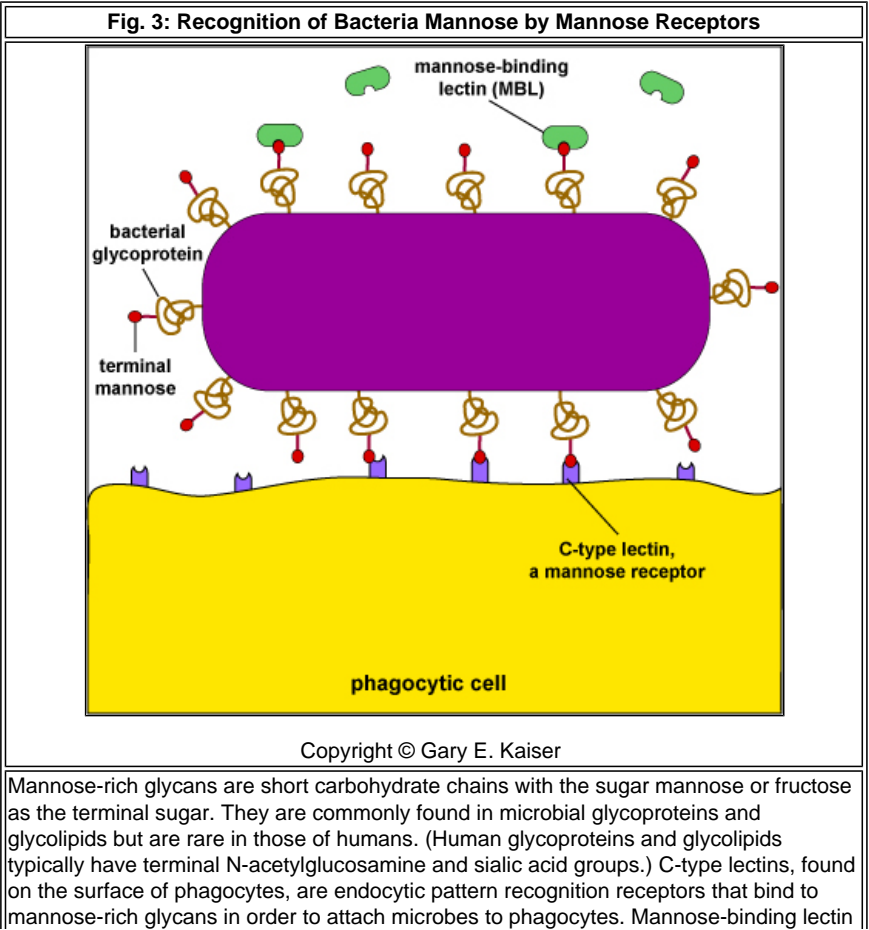
There are two functionally different major classes of pattern-recognition receptors: **endocytic pattern-recognition receptors** and **signaling pattern-recognition receptors**.

a. Endocytic (Phagocytic) Pattern-Recognition Receptors

Endocytic pattern-recognition receptors, also called phagocytic pattern-recognition receptors, are **found on the surface of phagocytes** and **promote the attachment of microorganisms to phagocytes** leading to their subsequent engulfment and destruction. They include:

1. Mannose receptors

Mannose receptors on the surface of phagocytes bind to various microbial carbohydrates such as those rich in **mannose or fucose**, and to **N-acetylglucosamine (NAG)**. Human glycoproteins and glycolipids typically have terminal N-acetylglucosamine and sialic acid groups. C-type lectins found on the surface of phagocytes are mannose receptors (**see Fig. 3**). It is now thought that mannose receptors may be quite important in removing potentially harmful mannose-containing glycoproteins such as lysosomal hydrolases that are produced in increased amounts during inflammation.



(MBL), also known as mannan-binding protein, is a soluble pattern recognition receptor in plasma and tissue fluid that binds to mannose-rich glycans on microbes in order to activate the lectin complement pathway.

2. Dectin-1

Dectin-1 recognizes beta-glucans (polymers of glucose) commonly found in fungal cell walls.

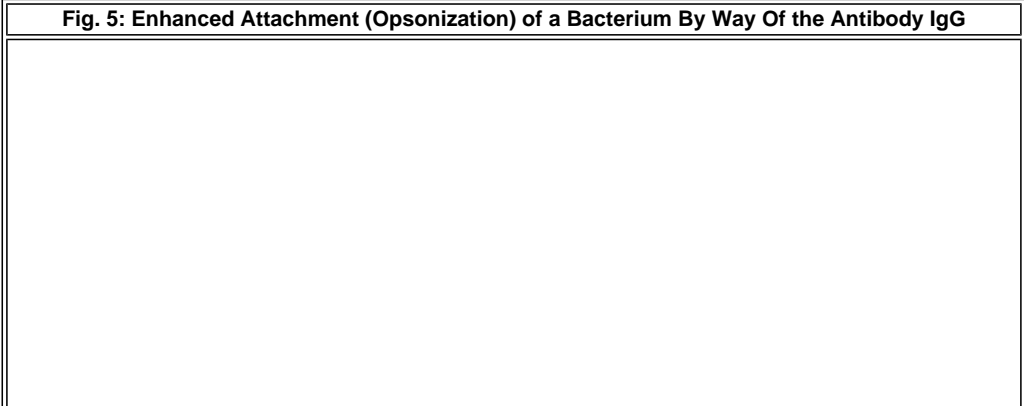
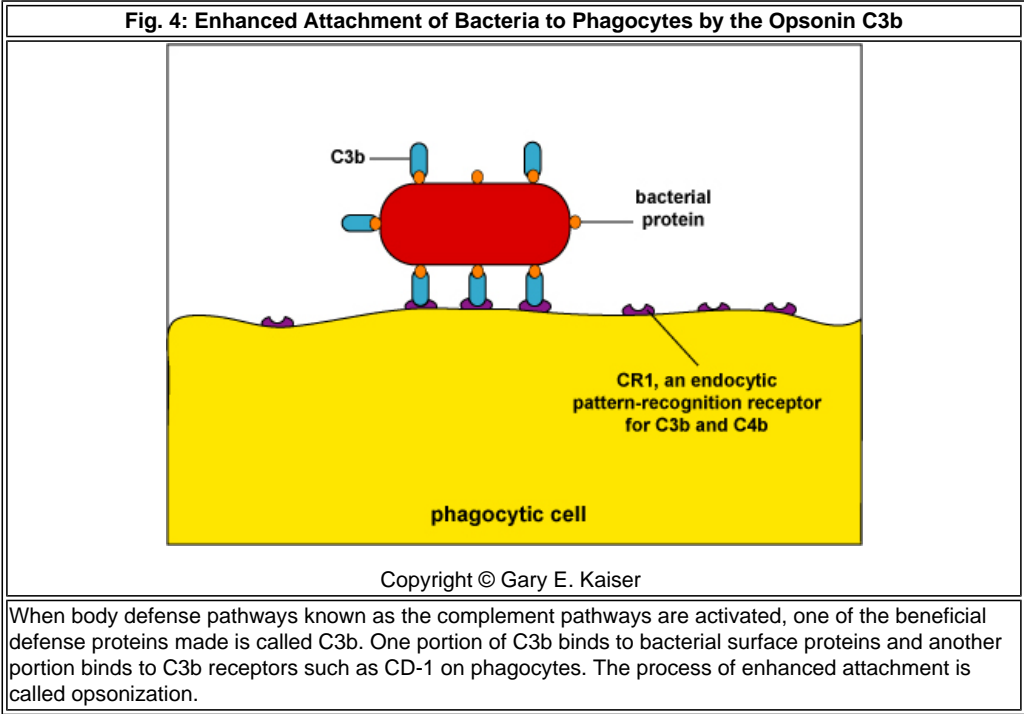
3. Scavenger receptors

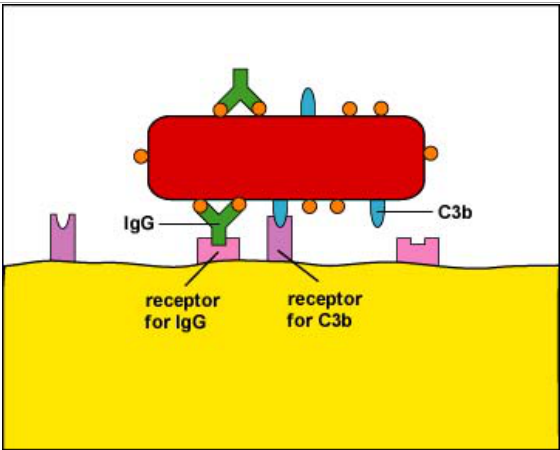
Scavenger receptors found on the surface of phagocytic cells bind to bacterial cell wall components such as LPS, peptidoglycan and teichoic acids (see Fig. 1). There are also scavenger receptors for certain components of other types of microorganisms, as well as for stressed, infected, or injured cells . Scavenger receptors include CD-36, CD-68, and SRB-1.

4. Opsonin receptors

Opsonins are soluble molecules produced as a part of the body's immune defenses that bind microbes to phagocytes. One portion of the opsonin binds to a PAMP on the microbial surface and another portion binds to a specific receptor on the phagocytic cell.

- Acute phase proteins circulating in the plasma, such as:
 - **mannose-binding lectin** (also called mannose-binding protein) that binds to various microbial carbohydrates such as those rich in **mannose or fucose**, and to **N-acetylglucosamine (NAG)**; and
 - **C-reactive protein (CRP)** that binds to phosphorylcholine portion of teichoic acids and lipopolysaccharides of bacterial and fungal cell walls. It also binds to the phosphocholine found on the surface of damaged or dead human cells.
- Complement pathway proteins, such as C3b (see Fig. 4) and C4b recognize a variety of PAMPS.
- Surfactant proteins in the alveoli of the lungs, such as SP-A and SP-D are opsonins.
- During adaptive immunity, the antibody molecule IgG can function as an opsonin (see Fig. 5).





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One of the functions of certain antibody molecules known as IgG is to stick antigens such as bacterial proteins and polysaccharides to phagocytes. The tips of the antibody, the Fab portion, have a shape that fits epitopes, portions of an antigen with a complementary shape. The stalk of the antibody is called the Fc portion and is able to bind to Fc receptors on phagocytes. Also, when body defense pathways known as the complement pathways are activated, one of the beneficial defense proteins made is called C3b. C3b binds by one end to bacterial surface proteins and by the other end to C3b receptors on phagocytes. The IgG and C3b are also known as opsonins and the process of enhanced attachment is also called opsonization.

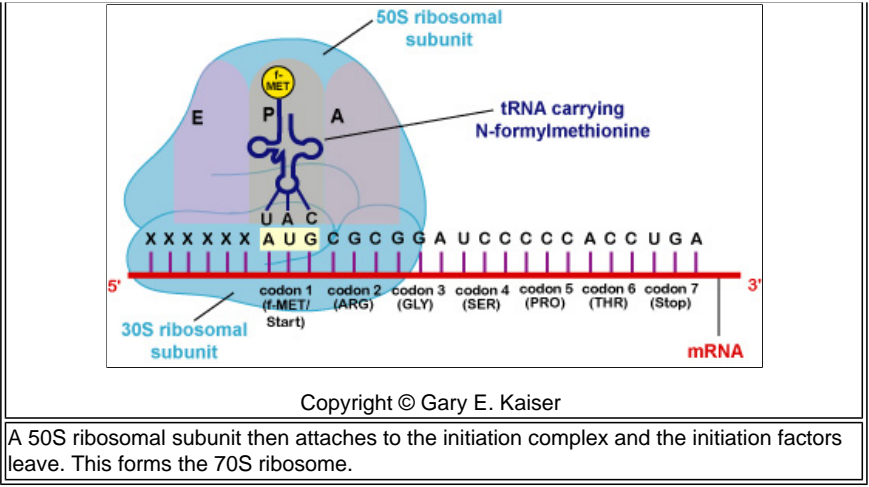
Flash animation illustrating the function of endocytic pattern-recognition receptors on phagocytes.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating the function of endocytic pattern-recognition receptors on phagocytes.
Glycoprotein molecules known as endocytic pattern-recognition receptors are found on the surface of phagocytes. They are so named because they recognize and bind to pathogen-associated molecular patterns - molecular components associated with microorganisms but not found as a part of eukaryotic cells. These include bacterial molecules such as peptidoglycan, lipoteichoic acids, mannans, and lipopolysaccharide (LPS). These receptors enable the phagocyte to attach to the cell wall of the microorganism so it can be engulfed and destroyed by lysosomes.

For more information: Preview of the acute phase response

5. *N*-formyl Met receptors

N-formyl methionine is the first amino acid produced in bacterial proteins since the f-met-tRNA in bacteria has an anticodon complementary to the AUG start codon (see Fig. 6). This form of the amino acid is not typically seen in mammalian proteins. FPR and FPRL1 are *N*-formyl receptors on neutrophils and macrophages. Binding of *N*-formyl Met to its receptor promotes the motility and the chemotaxis of these phagocytes. It also promotes phagocytosis.

Fig. 6: Translation of mRNA by tRNA: 50S Ribosomal Subunit Attaches to the Initiation Complex.



Signaling Pattern-Recognition Receptors

Signaling pattern-recognition receptors bind a number of microbial molecules: LPS, peptidoglycan, teichoic acids, flagellin, pilin, unmethylated cytosine-guanine dinucleotide or CpG sequences from bacterial and viral genomes; lipoteichoic acid, glycolipids, and zymosan from fungi; double-stranded viral RNA, and certain single-stranded viral RNAs. **Binding of microbial PAMPs to signaling PRRs promotes the production of:**

- inflammatory cytokines, such as such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-12 (IL-12);
- antiviral cytokines called type-1 interferons (IFN), such as IFN-alpha and IFN-beta;
- chemotactic factors, such as the chemokines interleukin-8 (IL-8), MCP-1, and RANTES; and
- antimicrobial peptides, such as human defensins and cathelicidins.

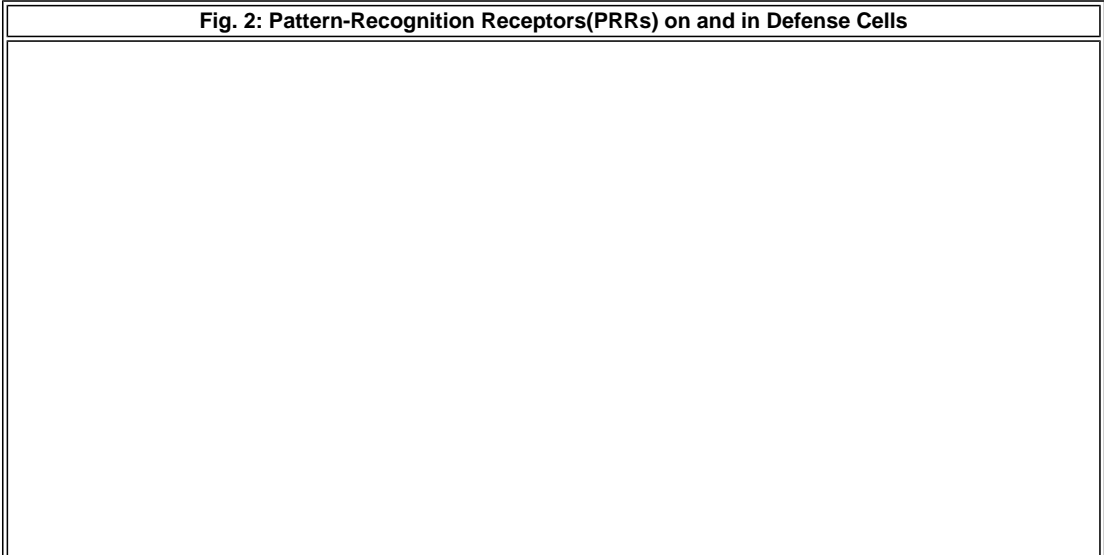
These molecules are crucial to initiating innate immunity and adaptive immunity.

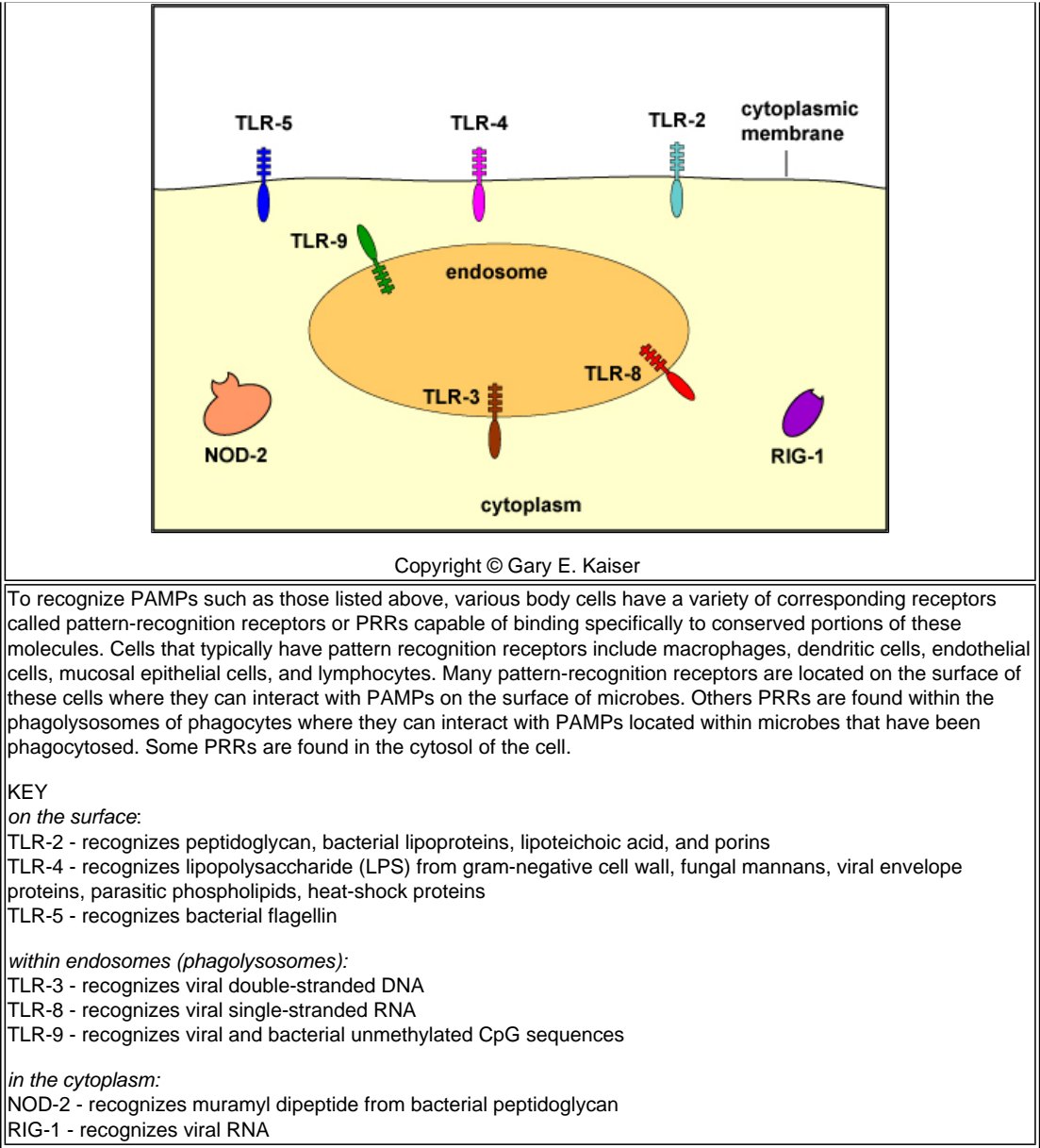
1. Signaling PRRs Found on Cell Surfaces (see Fig. 2)

A series of signaling pattern-recognition receptors known as toll-like receptors (TLRs) are found on the surface of a variety of defense cells and other cells. These TLRs play a major role in the induction of innate immunity and contribute to the induction of adaptive immunity.

Different combinations of TLRs appear in different cell types and may occur in pairs. Different TLRs directly or indirectly bind different microbial molecules. For example:

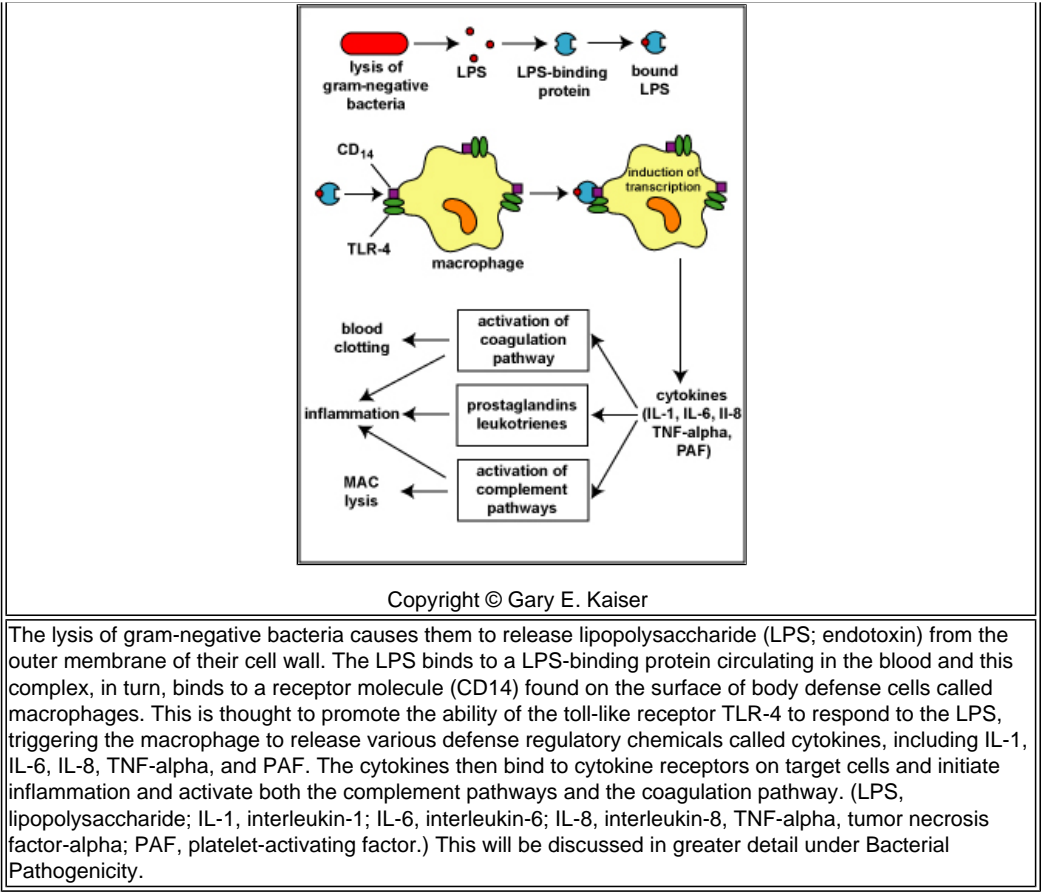
- a. TLR-2 - recognizes peptidoglycan, bacterial lipoproteins, lipoteichoic acid (Gram-positive bacteria), and porins (gram-negative bacteria).
- b. TLR-4 - recognizes lipopolysaccharide (Gram-negative bacteria), fungal mannans, viral envelope proteins, parasitic phospholipids, heat-shock proteins.
- c. TLR-5 - recognizes bacterial flagellin;
- d. TLR-1/TLR-2 pairs - binds to bacterial lipopeptides, lipomannans (mycobacteria) lipoteichoic acids (Gram-positive bacteria), cell wall beta glucans (bacteria and fungi), zymosan (fungi) and glycosylphosphatidylinositol (GPI)-anchored proteins (protozoa).
- e. TLR-2/TL6 pairs - also binds to bacterial lipopeptides, lipomannans (mycobacteria) lipoteichoic acids (Gram-positive bacteria), cell wall beta glucans (bacteria and fungi), zymosan (fungi) and glycosylphosphatidylinositol (GPI)-anchored proteins (protozoa).





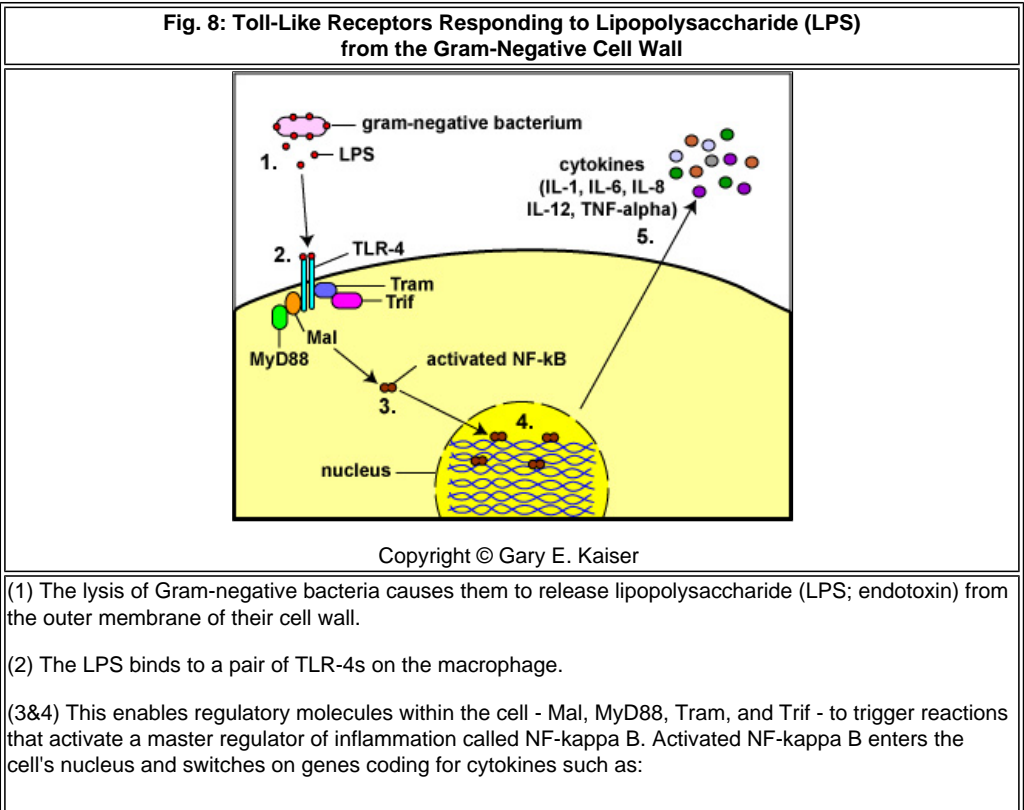
Many of the TLRs, especially those that bind to bacterial and fungal cell wall components, stimulate the transcription and translation of inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-12 (IL-12), as well as chemokines such as interleukin-8 (IL-8), MCP-1, and RANTES. These cytokines trigger innate immune defenses such as inflammation, fever, and phagocytosis in order to provide an immediate response against the invading microorganism (see Fig. 7). Because cytokines such as IL-1, TNF-alpha, and IL-12 that trigger an inflammatory response, they are often referred to as inflammatory cytokines. Chemokines are a group of cytokines that enable the **migration of leukocytes from the blood to the tissues** at the site of inflammation. To counter inflammation, anti-inflammatory cytokines such as IL-1 receptor antagonist, IL-4, and IL-10 are produced.

Fig. 7: Physiologic Action of Lipopolysaccharide (LPS) from the Gram-Negative Cell Wall



Another cell surface PRR is CD14. CD14 is found on monocytes, macrophages, and neutrophils and promotes the ability of TLR-4 to respond to LPS. LPS typically binds to LPS-binding protein in the plasma and tissue fluid. The LPS-binding protein promotes the binding of LPS to the CD14 receptors. At that point the LPS-binding protein comes off and the LPS-CD14 bind to TLR-4. Interaction of LPS and CD14 with TLR-4 leads to an elevated synthesis and secretion of inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-alpha, and platelet-activating factor (PAF). These cytokines then bind to cytokine receptors on target cells and initiate inflammation and activate both the complement pathways and the coagulation pathway (see Fig. 7).

The signaling process for the CD14 and TLR-4 response to LPS is shown in Fig. 8.



- a. Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-alpha): enhance inflammatory responses;
- b. Interleukin-8 (IL-8): aids in the ability of white blood cells to leave the blood vessels and enter the tissue; a chemoattractant for phagocytes;
- c. Interleukin-6 (IL-6) promotes B-lymphocyte activity; and
- d. Interleukin-12 (IL-12): promotes T-lymphocyte activity. (5)

Flash animation illustrating signaling toll-like receptors on defense cells: LPS and TLR-4.
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html5 version of animation for iPad illustrating signaling toll-like receptors on defense cells: LPS and TLR-4.
<p>1) Gram-negative bacteria release lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall.</p> <p>2) The LPS binds to a pair of TLR-4s on defense cells such as macrophages and dendritic cells. LPS also binds to LPS-binding protein in the plasma and tissue fluid. The LPS-binding protein promotes the binding of LPS to the CD14 receptors. At that point the LPS-binding protein comes off and the LPS-CD14 bind to TLR-4.</p> <p>3) The binding of LPS to TLR-4 enables regulatory molecules within the cell - Mal, MyD88, Tram, and Trif - to trigger reactions that activate a master regulator of inflammation called NF-kappa B. Activated NF-kappa B enters the cell's nucleus and switches on genes coding for cytokines such as:</p> <ul style="list-style-type: none">a. Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-alpha): enhance inflammatory responses;b. Interleukin-8 (IL-8): aids in the ability of white blood cells to leave the blood vessels and enter the tissue; a chemoattractant for phagocytes;c. Interleukin-6 (IL-6) promotes B-lymphocyte activity; andd. Interleukin-12 (IL-12): promotes T-lymphocyte activity. (5) <p>4) Cytokine genes are transcribed into mRNA molecules that go to the cytoplasm to be translated into inflammatory cytokines that are subsequently secreted from the cell.</p>

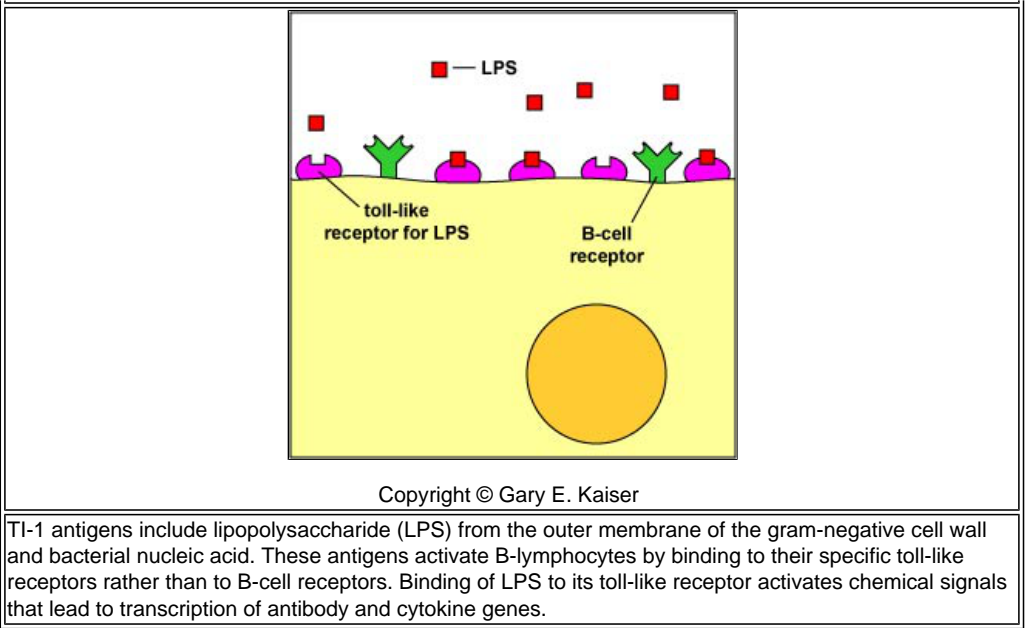
Flash animation illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6.
<p>1) Gram-positive bacteria release lipoteichoic acid (LTA) from their cell wall.</p> <p>2) The LTA binds to a TLR-2/TLR-6 pair on defense cells such as macrophages and dendritic cells.</p> <p>3) The binding of LTA to TLR-2/TLR-6 enables regulatory molecules within the cell - Mal, MyD88, Tram, and Trif - to trigger reactions that activate a master regulator of inflammation called NF-kappa B. Activated NF-kappa B enters the cell's nucleus and switches on genes coding for cytokines such as:</p> <ul style="list-style-type: none">a. Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-alpha): enhance inflammatory responses;b. Interleukin-8 (IL-8): aids in the ability of white blood cells to leave the blood vessels and enter the tissue; a chemoattractant for phagocytes;c. Interleukin-6 (IL-6) promotes B-lymphocyte activity; andd. Interleukin-12 (IL-12): promotes T-lymphocyte activity. (5) <p>4) Cytokine genes are transcribed into mRNA molecules that go to the cytoplasm to be translated into inflammatory cytokines that are subsequently secreted from the cell.</p>

For more information: Preview of inflammation

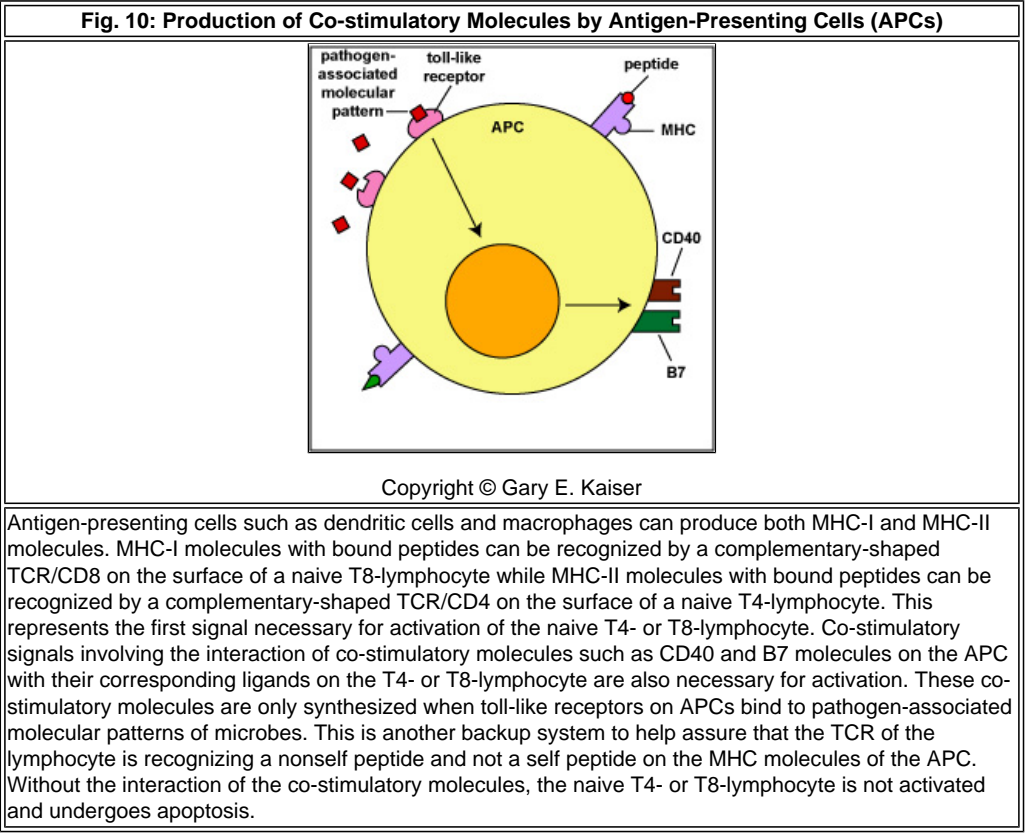
TLRs also participate in **adaptive immunity** by triggering various secondary signals needed for humoral immunity (the production of antibodies) and cell-mediated immunity (the production of cytotoxic T-lymphocytes, activated macrophages, and additional cytokines). Without innate immune responses there could be no adaptive immunity.

a. T-independent (TI) antigens allow B-lymphocytes to mount an antibody response without the requirement of interaction with effector T4-lymphocytes. The resulting antibody molecules are generally of the IgM isotype and do not give rise to a memory response. There are two basic types of T-independent antigens: TI-1 and TI-2. TI-1 antigens are pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) from the outer membrane of the gram-negative cell wall and lipoteichoic acids from the gram-positive cell wall. These antigens activate B-lymphocytes by binding to their specific toll-like receptors rather than to B-cell receptors (see Fig. 9). Antibody molecules generated against TI-1 antigens are often called "natural antibodies" because they are always being made against bacteria present in the body.

Fig. 9: Activation of a B-lymphocyte by way of LPS Binding to Toll-like Receptors



b. The activation of naive T-lymphocytes requires co-stimulatory signals involving the interaction of accessory molecules on antigen-presenting cells or APCs with their corresponding ligands on T-lymphocytes. These co-stimulatory molecules are only synthesized when toll-like receptors on APCs bind to pathogen-associated molecular patterns of microbes (see Fig. 10).



2. Signaling PRRs Found in the Membranes of the Endosomes

Signaling PRRs found in the membranes of the endosomes (phagolysosomes) used to degrade pathogens (see Fig. 2):

- a. TLR-3 - binds double-stranded viral RNA;
- b. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;
- c. TLR-8 - binds single-stranded viral RNA;
- d. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommon or masked in human DNA and RNA.

The diagram illustrates the localization of various Toll-like receptors (TLRs) and other pattern recognition receptors within a cell. The cytoplasmic membrane is shown at the top. TLR-5 (blue), TLR-4 (pink), and TLR-2 (teal) are embedded in the membrane. TLR-9 (green) is located on the endosome membrane. TLR-3 (brown) and TLR-8 (red) are in the cytoplasm. NOD-2 (orange) and RIG-1 (purple) are also in the cytoplasm.

To recognize PAMPs such as those listed above, various body cells have a variety of corresponding receptors called pattern-recognition receptors or PRRs capable of binding specifically to conserved portions of these molecules. Cells that typically have pattern recognition receptors include macrophages, dendritic cells, endothelial cells, mucosal epithelial cells, and lymphocytes. Many pattern-recognition receptors are located on the surface of these cells where they can interact with PAMPs on the surface of microbes. Others PRRs are found within the phagolysosomes of phagocytes where they can interact with PAMPs located within microbes that have been phagocytosed. Some PRRs are found in the cytosol of the cell.

on the surface:

TLR-4 - recognizes lipopolysaccharide (LPS) from gram-negative cell wall, fungal mannans, viral envelope proteins, parasitic phospholipids, heat-shock proteins

TLR-5 - recognizes bacterial flagellin

TLR-3 - recognizes viral double-stranded DNA

TLR-9 - recognizes viral and bacterial unmethylated CpG motifs

RIG-1 - recognizes viral RNA

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In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by recognizing molecules unique to microorganisms that are not associated with human cells. These unique molecules are called pathogen-associated molecular patterns or PAMPs and they bind to pattern recognition receptors called toll-like receptors (TLRs) found on host defense cells. For example, most viral genomes contain a high frequency of unmethylated cytosine-guanine dinucleotide sequences (a cytosine lacking a methyl or CH₃ group and located adjacent to a guanine). Mammalian DNA has a low frequency of cytosine-guanine dinucleotides and most are methylated. In addition, most viruses produce unique double-stranded viral RNA, and some viruses produce uracil-rich single-stranded viral RNA during portions of their life cycle. The binding of these unique viral molecules bind to the endosomal TLRs of defense cells such as macrophages and dendritic cells triggers the production of antiviral cytokines called type I interferons that are able to block viral replication.

- a. TLR-3 - binds double-stranded viral RNA;
- b. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;
- c. TLR-8 - binds single-stranded viral RNA;
- d. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommon or masked in human DNA and RNA.

Interferons induce uninfected cells to produce enzymes capable of degrading mRNA. These enzymes remain inactive until the uninfected cell becomes infected with a virus. At this point, the enzymes are activated and begin to degrade both viral and cellular mRNA. This not only blocks viral protein synthesis, it also eventually kills the infected cell.

GIF animation showing the antiviral nature of interferon

3. Signaling PRRs and DRRs Found in the Cytoplasm

Pattern-recognition receptors or PRRs found in the cytoplasm (see Fig. 2) include:

- a. **NODs** (nucleotide-binding oligomerization domain)

NOD proteins, including NOD-1 and NOD-2, are cytosolic proteins that allow intracellular recognition of peptidoglycan components.

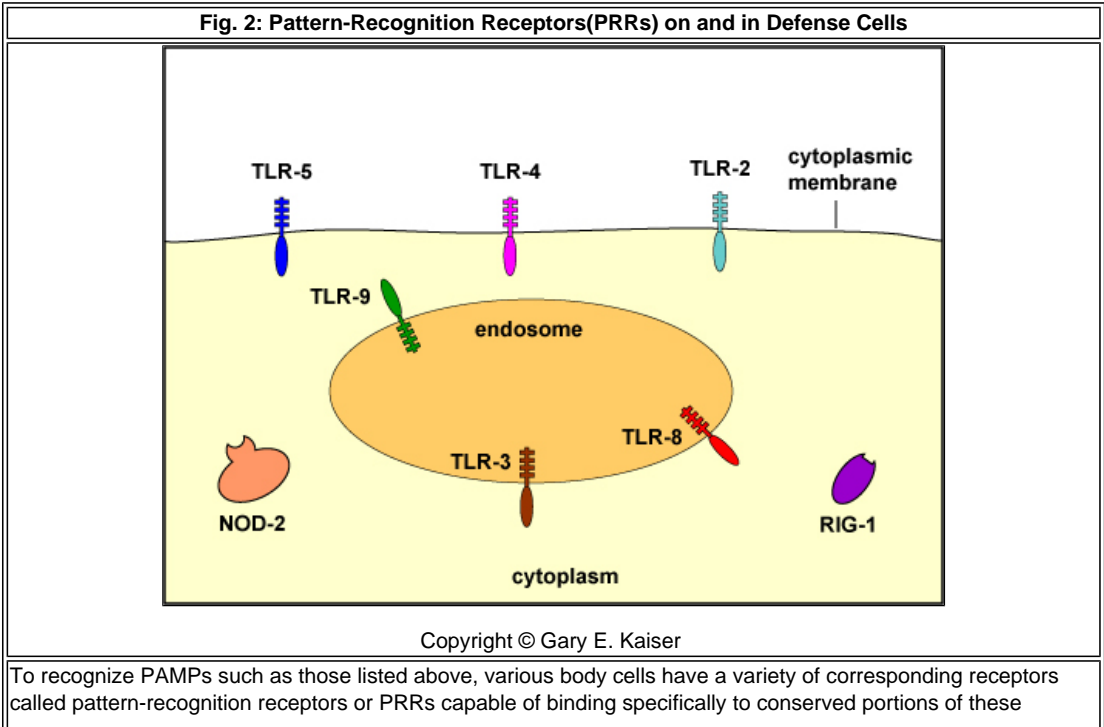
- 1. NOD-1 recognizes peptidoglycan containing the muramyl dipeptide NAG-NAM-gamma-D-glutamyl-meso diaminopimelic acid, part of the peptidoglycan monomer in common gram-negative bacteria and just a few gram-positive bacteria.
- 2. NOD-2 recognizes peptidoglycan containing the muramyl dipeptide NAG-NAM-L-alanyl-isoglutamine found in practically all bacteria (see Fig. 2).

As macrophages phagocytose either whole bacteria or peptidoglycan fragments released during bacterial growth, the peptidoglycan is broken down into muramyl dipeptides. Binding of the muramyl dipeptides to NOD-1 or NOD-2 leads to the activation of genes coding for inflammatory cytokines such as IL-1, TNF-alpha, IL-8, and IL-12 in a manner similar to the cell surface TLRs. Activation of NOD-2 also induces the production of antimicrobial peptides such as defensins as well as microbicidal reactive oxygen species (ROS).

- b. **CARD-containing proteins**

CARD (caspase activating and recruitment domain)-containing proteins, such as RIG-1 (retinoic acid-inducible gene-1) and MDA-5 (melanoma differentiation-associated gene-5), are cytoplasmic sensors of viral RNA molecules that trigger the synthesis of type-1 interferons, antiviral cytokines that block viral replication within infected host cells in a manner similar to the endosomal TLRs. RIG-1 recognizes 5'-PPPs on viral RNAs. The 5'-PPPs on host cell RNAs are either capped or removed and are not recognized by RIG-1.

Rig-1 and MDA-5 can also, through another regulatory pathway, stimulate the production of inflammatory cytokines.



molecules. Cells that typically have pattern recognition receptors include macrophages, dendritic cells, endothelial cells, mucosal epithelial cells, and lymphocytes. Many pattern-recognition receptors are located on the surface of these cells where they can interact with PAMPs on the surface of microbes. Others PRRs are found within the phagolysosomes of phagocytes where they can interact with PAMPs located within microbes that have been phagocytosed. Some PRRs are found in the cytosol of the cell.

KEY

on the surface:

TLR-2 - recognizes peptidoglycan, bacterial lipoproteins, lipoteichoic acid, and porins

TLR-4 - recognizes lipopolysaccharide (LPS) from gram-negative cell wall, fungal mannans, viral envelope proteins, parasitic phospholipids, heat-shock proteins

TLR-5 - recognizes bacterial flagellin

within endosomes (phagolysosomes):

TLR-3 - recognizes viral double-stranded DNA

TLR-8 - recognizes viral single-stranded RNA

TLR-9 - recognizes viral and bacterial unmethylated CpG sequences

in the cytoplasm:

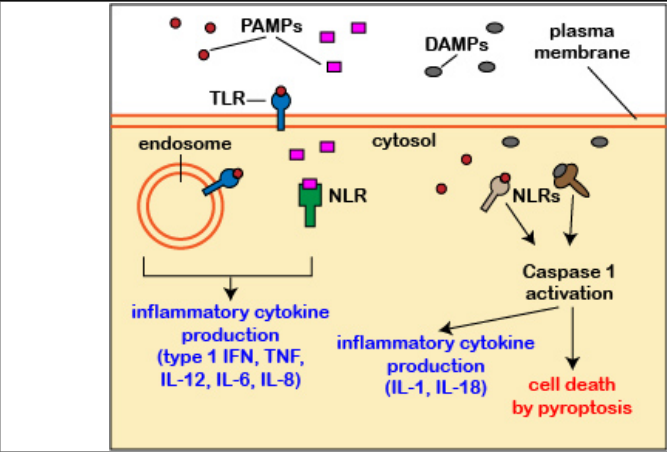
NOD-2 - recognizes muramyl dipeptide from bacterial peptidoglycan

RIG-1 - recognizes viral RNA

Detection of PAMPs by PRRs in the cytosol trigger the formation of multi-protein complexes called inflammasomes which, in turn, leads to the activation of caspase-1. Caspase-1 triggers the formation of inflammatory cytokines and can also result in an inflammatory response-induced cell suicide called pyroptosis. Pyroptosis, unlike apoptosis, leads to the release of PAMPs as well as inflammatory cytokines from the lysed cell.

Pyroptosis is initiated by **PAMPs binding to pattern-recognition receptors (PRRs)** on various defense cells which then **triggers the production of inflammatory cytokines and type-1 interferons**. Other PRRs, called nod-like receptors (NLRs) located in the cytosol of these defense cells recognize PAMPs and DAMPs that have entered the host cell's cytosol. Some NLRs trigger the production of inflammatory cytokines while others activate caspase 1-dependent pyroptosis of the cell causing the release of its intracellular inflammatory cytokines (**see Fig. 3**). The binding of PAMPs or DAMPs to their respective NLRs triggers the assembly of multiprotein complexes called inflammasomes in the cytosol of the host cell. It is these inflammasomes that activate caspase 1 and induce inflammation and pyroptosis. **Pyroptosis results in production of proinflammatory cytokines, rupture of the cell's plasma membrane, and subsequent release of proinflammatory intracellular contents**. It plays an **essential role in innate immunity** by promoting inflammation to control microbial infections. At **highly elevated levels, however, it can cause considerable harm** to the body and even death.

Fig. 3: Activation of Pyroptosis



Pyroptosis is initiated by PAMPs binding to pattern-recognition receptors (PRRs) on various defense cells, such as the Toll-like receptors (TLR) shown here. This, in turn, triggers the production of type-1 interferons and inflammatory cytokines such as TNF, IL-12, IL-6, and IL-8. Other PRRs, called nod-like receptors (NLRs) located in the cytosol of these defense cells recognize PAMPs and DAMPs that have entered the host cell's cytosol. Some NLRs trigger the production of inflammatory cytokines such as IL-1 and IL-18 while others activate caspase 1-dependent pyroptosis of the cell causing the release of its intracellular inflammatory cytokines.

(While not shown here, the binding of PAMPs or DAMPs to their respective NLRs triggers the assembly of multiprotein complexes called inflammasomes in the cytosol of the host cell. It is these inflammasomes that activate caspase 1 and induce inflammation and pyroptosis.)

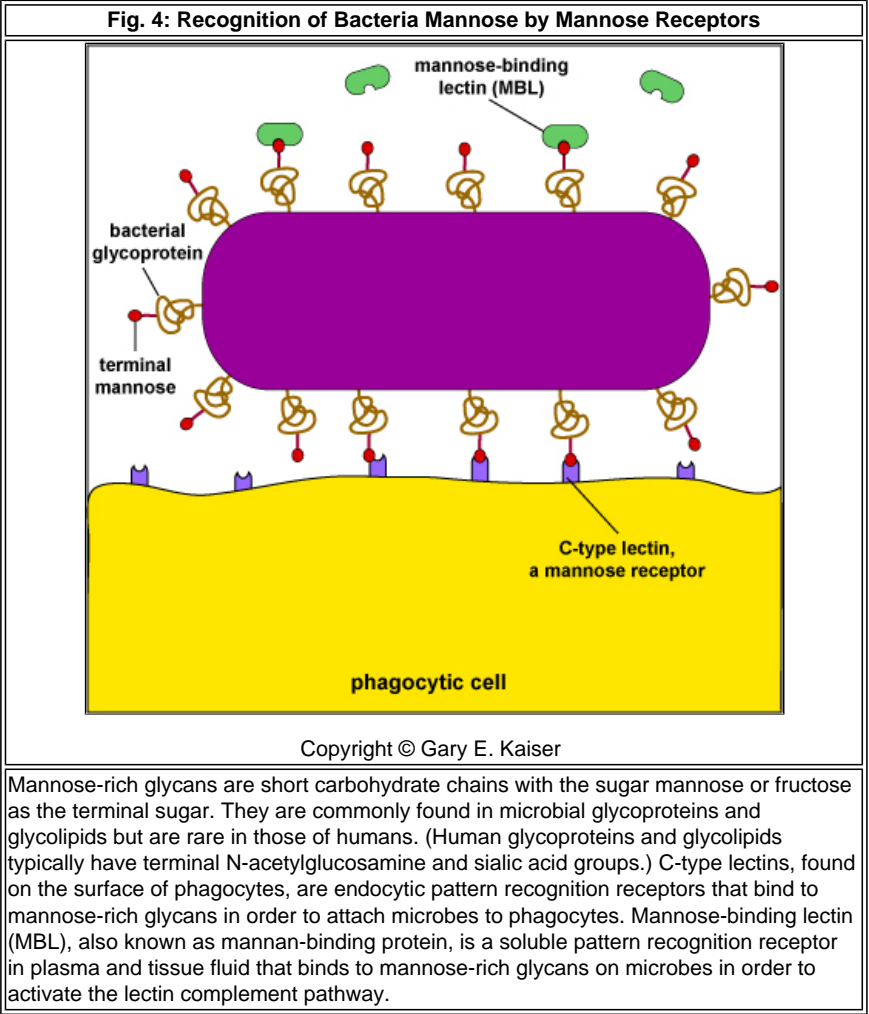
c. Danger recognition receptors or DRRs

Danger recognition receptors or DRRs found in the cytoplasm **recognize danger-associated molecular patterns (DAMPs)** in the cytosol such as altered membrane phospholipids, and materials released from damaged phagosomes and damaged lysosomes, including antibodies bound to microbes from opsonization. DAMPs are also produced as a result of tissue injury during cancer, heart attack, and stroke. Detection of DAMPs by DRRs in the cytosol also **triggers the activation of inflammasomes, release of inflammatory cytokines, and pyroptosis.**

4. Secreted Signaling PRRs Found in Plasma and Tissue Fluid

In addition to the PRRs found on or within cells, there are also **secreted pattern-recognition receptors**. These PRRs bind to microbial cell walls and enable them to activate **the complement pathways**, as well as by phagocytes. For example, mannan-binding lectin -also known as mannan-binding protein - is synthesized by the liver and released into the bloodstream as part of the acute phase response discussed later in Unit 4. Here it can bind to the carbohydrates on bacteria, yeast, some viruses, and some parasites (**see Fig. 4**). This, in turn, activates the lectin complement pathway (discussed later in Unit 4) and results in the production of a variety of activated complement proteins that are able to trigger inflammation, chemotactically attract phagocytes to the infection site, promote the attachment of antigens to phagocytes via enhanced attachment or opsonization, and cause lysis of gram-negative bacteria and infected or transformed human cells.

Other secreted PRRs include C-reactive protein (CRP), surfactant protein A (SP-A), surfactant protein D (SP-D), collectin liver 1 (CL-L1), and ficolins.



Flash animation showing activation of the lectin pathway, formation of C3 convertase, and formation of C5 convertase.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing activation of the lectin pathway, formation of C3 convertase, and formation of C5 convertase.
The lectin pathway is activated by the interaction of various microbial carbohydrates such as mannose, fucose, N-acetylglucosamine (NAG), lipoteichoic acids, and lipopolysaccharide (LPS) with mannose-binding lectin (MBL) or ficolins which forms complexes with two proteases called MASP1 and MASP2 in the plasma. (Lectins are carbohydrate-binding proteins.) The binding of the MBL (or the ficolin) to the microbial carbohydrate activates the associated MASP2 giving it the enzymatic activity to split C4 into C4a and C4b, as well as C2 into C2a and C2b.

TPS Questions

Concept Maps for PRRs and DRRs

Self Quiz for Pattern-Recognition Receptors (PRRs) and Danger-Recognition Receptors (DRRs)

Quiz Group

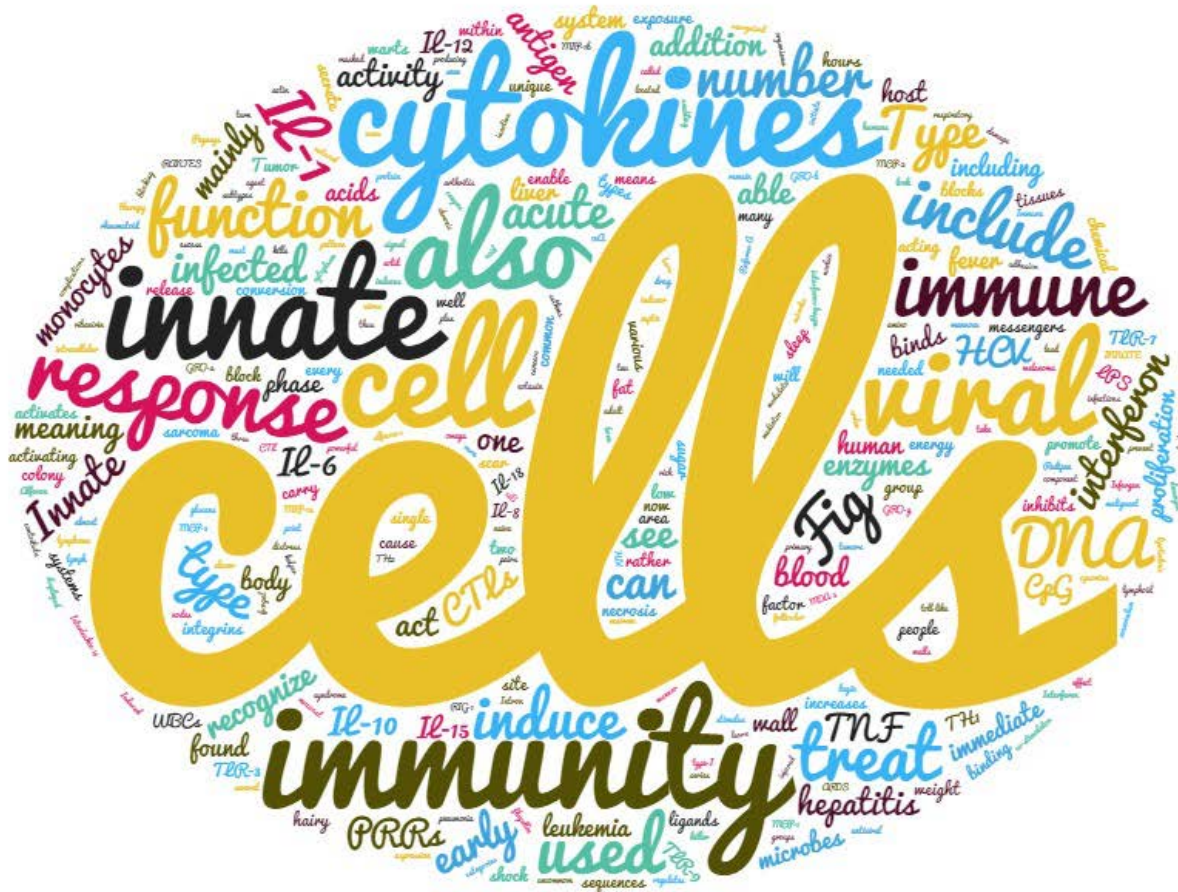


Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

Innate Immunity

Early Induced Innate Immunity: Cytokines Important in Innate Immunity



1. Cytokines are low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems.
2. Cytokines are pleiotropic, meaning meaning that a particular cytokine can act on a number of different types of cells rather than a single cell type.
3. Cytokines are redundant, meaning that a number of different cytokines are able to carry out the same function.
4. Cytokines are multifunctional, meaning that the same cytokine is able to regulate a number of different functions.
5. Tumor necrosis factor-alpha (TNF- α) and interleukin-1 (IL-1) are the principle cytokines that mediates acute inflammation.
6. Chemokines are a group of cytokines that enable the migration of leukocytes from the blood to the tissues at the site of inflammation.
7. Type I interferons, produced abundantly by plasmacytoid dendritic cells, by virtually any virus-infected cell, and by other defense cells provide an early innate immune response against viruses by inducing uninfected cells to produce enzymes capable of degrading viral mRNA and blocking translation in eukaryotic cells. They also enhancing the activities of CTLs, macrophages, dendritic cells, NK cells, and antibody-producing cells and induce chemokine production to attract leukocytes to the area.
8. Type II interferon is involved in stimulating an inflammatory response.

Common Course Objective

1. Explain what cytokines are and their role in immunity.

2. Recall the different types of cytokines and predict what type of response would occur when released.

Detailed Learning Objectives

- 1*. Describe the following:
- a. cytokines
 - b. chemokines
 - c. interferons
2. State what is meant by the phrase "Cytokines are pleiotropic, redundant, and multifunctional."
3. Name the two cytokines that are most important in stimulating acute inflammation.
- 4*. Describe specifically how type I interferons are able to block viral replication within an infected host cell.
5. Describe how an overactive TLR-4 receptor can increase the risk of SIRS in a person if Gram-negative bacteria enter the bloodstream.
6. Briefly describe two specific examples of how an improper functioning PRR can lead to an increased risk of a specific infection or disease.

(*) = Common theme throughout the course

TPS Question

Cytokines Important in Innate Immunity

Cytokines are low molecular weight, soluble proteins that are produced in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems. They are produced by virtually all cells involved in innate and adaptive immunity, but especially by T- helper (TH) lymphocytes. The activation of cytokine-producing cells triggers them to synthesize and secrete their cytokines. The cytokines, in turn, are then able to bind to specific cytokine receptors on other cells of the immune system and influence their activity in some manner.

Cytokines are pleiotropic, redundant, and multifunctional.

- **Pleiotropic** means that a particular cytokine can act on a number of different types of cells rather than a single cell type.
- **Redundant** refers to the ability of a number of different cytokines to carry out the same function.
- **Multifunctional** means the same cytokine is able to regulate a number of different functions.

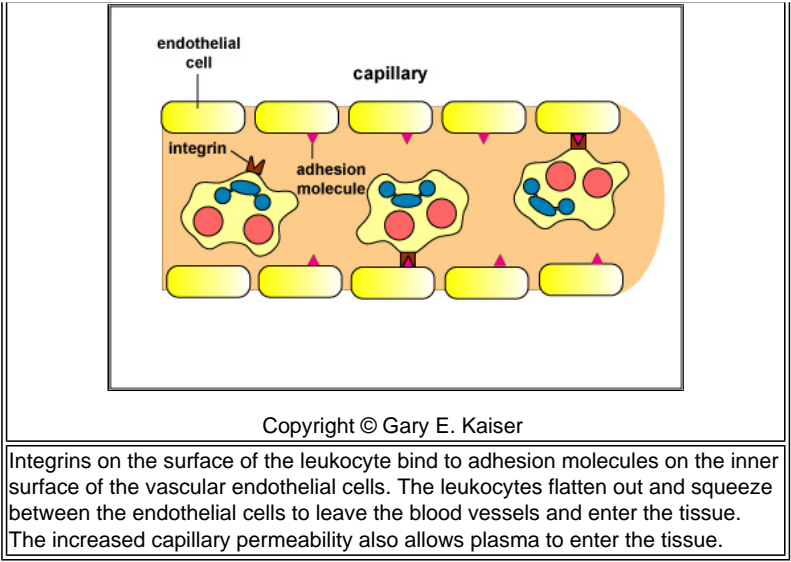
Some cytokines are antagonistic in that one cytokine stimulates a particular defense function while another cytokine inhibits that function. Other cytokines are synergistic wherein two different cytokines have a greater effect in combination than either of the two would by themselves.

There are **three functional categories of cytokines**:

1. cytokines that regulate innate immune responses,
2. cytokines that regulate adaptive Immune responses, and
3. cytokines that stimulate hematopoiesis.

Cytokines that regulate innate immunity are produced primarily by mononuclear phagocytes such as macrophages and dendritic cells, although they can also be produced by T-lymphocytes, NK cells, endothelial cells, and mucosal epithelial cells. They are **produced primarily in response to pathogen-associated molecular patterns (PAMPs)** such as LPS, peptidoglycan monomers, teichoic acids, unmethylated cytosine-guanine dinucleotide or CpG sequences in bacterial and viral genomes, and double-stranded viral RNA. Cytokines produced in response to PRRs on cell surfaces, such as the inflammatory cytokines TNF-alpha, IL-1, IL-6, and IL-8, mainly act on leukocytes and the endothelial cells that form blood vessels in order to promote and control early inflammatory responses (**see Fig. 1**). Cytokines produced in response to PRRs that recognize viral nucleic acids, such as type I interferons, primarily block viral replication within infected host cells.

Fig. 1: Diapedesis During Inflammation



Examples include:

a. **Tumor necrosis factor-alpha (TNF-a)**

TNF-a is the principle cytokine that mediates acute inflammation. In excessive amounts it also is the principal cause of systemic complications such as the shock cascade. Functions include acting on endothelial cells to stimulate inflammation and the coagulation pathway; stimulating endothelial cells to produce selectins and ligands for leukocyte integrins during diapedesis; stimulating endothelial cells and macrophages to produce chemokines that contribute to diapedesis, chemotaxis, and the recruitment of leukocytes; stimulating macrophages to secrete interleukin-1 (IL-1) for redundancy; activating neutrophils and promoting extracellular killing by neutrophils; stimulating the liver to produce acute phase proteins, and acting on muscles and fat to stimulate catabolism for energy conversion. TNF-a stimulates the endothelial cells that form capillaries to express proteins that activate blood clot formation within the capillaries. This occludes local blood flow to help prevent microbes from entering the bloodstream. In addition, TNF is cytotoxic for some tumor cells; interacts with the hypothalamus to induce fever and sleep; stimulates the synthesis of collagen and collagenase for scar tissue formation; and activates macrophages. TNF is produced by monocytes, macrophages, dendritic cells, T_H1 cells, and other cells.

b. **Interleukin-1 (IL-1)**

IL-1 function similarly to

TNF-a in that it mediates acute inflammatory responses. It also works synergistically with TNF to enhance inflammation. Functions of IL-1 include promoting inflammation; activating the coagulation pathway, stimulating the liver to produce acute phase proteins, catabolism of fat for energy conversion, inducing fever and sleep; stimulates the synthesis of collagen and collagenase for scar tissue formation; stimulates the synthesis of adhesion factors on endothelial cells and leukocytes (**see Fig. 1**) for diapedesis; and activates macrophages. IL-1 is produced primarily by monocytes, macrophages, dendritic cells, endothelial cells, and some epithelial cell.

c. **Chemokines**

Chemokines are a group of cytokines that enable the **migration of leukocytes from the blood to the tissues at the site of inflammation.** They increase the affinity of integrins on leukocytes for ligands on the vascular wall (**see Fig. 1**) during diapedesis, regulate the polymerization and depolymerization of actin in leukocytes for movement and migration, and function as chemoattractants for leukocytes. In addition, they trigger some WBCs to release their killing agents for extracellular killing and induce some WBCs to ingest the remains of damaged tissue. Certain chemokines promote angiogenesis. Chemokines also regulate the movement of B-lymphocytes, T-lymphocytes, and dendritic cells through the lymph nodes and the spleen. When produced in excess amounts, chemokines can lead to damage of healthy tissue as seen in such disorders as rheumatoid arthritis, pneumonia, asthma, adult respiratory distress syndrome (ARDS), and septic shock. Examples of chemokines include IL-8, MIP-1a, MIP-1b, MCP-1, MCP-2, MCP-3, GRO-a, GRO-b, GRO-g, RANTES, and eotaxin. Chemokines are produced by many cells including leukocytes, endothelial cells, epithelial cells, and fibroblasts.

d. **Interleukin-12 (IL-12)**

IL-12 is a primary mediator of early innate immune responses to intracellular microbes. It is also an inducer of cell-mediated immunity. It functions to stimulate the synthesis of interferon-gamma by T-lymphocytes and NK cells; increases the killing activity of cytotoxic T-lymphocytes and NK cells; and stimulates the differentiation of naive T4-lymphocytes into interferon-gamma producing T_H1 cells. It is produced mainly by macrophages and dendritic cells.

e. **Type I Interferons**

Interferons modulate the activity of virtually every component of the immune system. **Type I interferons** include 13 subtypes of interferon-alpha, interferon-beta, interferon omega, interferon-kappa, and interferon tau. (There is only one **type II interferon**, interferon-gamma, which is involved in the inflammatory response.)

The most powerful stimulus for type I interferons is the binding of viral DNA or RNA to toll-like receptors TLR-3, TLR-7, and TLR-9 in endosomal membranes.

1. TLR-3 - binds double-stranded viral RNA;
2. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;
3. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommon or masked in human DNA and RNA.

Flash animation showing toll-like receptors (TLRs) recognizing viral double-stranded RNA.

Copyright © Gary E. Kaiser

html5 version of animation for iPad showing toll-like receptors (TLRs) recognizing viral double-stranded RNA.

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by recognizing molecules unique to microorganisms that are not associated with human cells. These unique molecules are called pathogen-associated molecular patterns or PAMPS and they bind to pattern recognition receptors called toll-like receptors (TLRs) found on host defense cells. For example, most viral genomes contain a high frequency of unmethylated cytosine-guanine dinucleotide sequences (a cytosine lacking a methyl or CH₃ group and located adjacent to a guanine). Mammalian DNA has a low frequency of cytosine-guanine dinucleotides and most are methylated. In addition, most viruses produce unique double-stranded viral RNA, and some viruses produce uracil-rich single-stranded viral RNA during portions of their life cycle. The binding of these unique viral molecules bind to the endosomal TLRs of defense cells such as macrophages and dendritic cells triggers the production of antiviral cytokines called type I interferons that are able to block viral replication.

a. TLR-3 - binds double-stranded viral RNA;

b. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;

c. TLR-8 - binds single-stranded viral RNA;

d. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommon or masked in human DNA and RNA.

Interferons induce uninfected cells to produce enzymes capable of degrading mRNA. These enzymes remain inactive until the uninfected cell becomes infected with a virus. At this point, the enzymes are activated and begin to degrade both viral and cellular mRNA. This not only blocks viral protein synthesis, it also eventually kills the infected cell.

Signaling pattern recognition receptors located in the cytoplasm of cells such as RIG-1 and MDA-5 also signal synthesis and secretion of type-I interferons.

For more information: Review of pattern-recognition receptors (PRRs)

Type I interferons, produced abundantly by plasmacytoid dendritic cells, by virtually any virus-infected cell, and by other defense cells provide an early innate immune response against viruses. **Interferons induce uninfected cells to produce an enzyme capable of degrading viral mRNA**, as well as one that blocks translation in eukaryotic cells. These enzymes remain inactive until the uninfected cell becomes infected with a virus. At this point, the enzymes are activated and begin to **degrade viral mRNA** and block translation in the host cell. This not only **blocks viral protein synthesis**, it also eventually **kills the infected cell (see Slideshow Fig. 2A and Fig. 2B)**. In addition, type I interferons also cause infected cells to produce enzymes that interfere with transcription of viral RNA or DNA. They also promote body defenses by enhancing the activities of CTLs, macrophages, dendritic cells, NK cells, and antibody-producing cells, as well as induce chemokine production to attract leukocytes to the area.

Slideshow

Activity

GIF animation showing the antiviral nature of interferon.

Type I interferons also induce MHC-I antigen expression needed for recognition of antigens by cytotoxic T-lymphocytes; augment macrophages, NK cells, cytotoxic T-lymphocytes, and B-lymphocytes activity; and induce fever. Interferon-alpha is produced by T-lymphocytes, B-lymphocytes, NK cells, monocytes/macrophages; interferon-beta by virus-infected cells, fibroblasts, macrophages, epithelial cells, and endothelial cells.

f. Interleukin-6 (IL-6)

IL-6 functions to stimulate the liver to produce acute phase proteins; stimulates the proliferation of B-lymphocytes; and increases neutrophil production. IL-6 is produced by many cells including T-lymphocytes, macrophages, monocytes, endothelial cells, and fibroblasts.

g. Interleukin-10 (IL-10)

IL-10 is an inhibitor of activated macrophages and dendritic cells and as such, regulates innate immunity and cell-mediated immunity. IL-10 inhibits their production of IL-12, co-stimulator molecules, and MHC-II molecules, all of which are needed for cell-mediated immunity. IL-10 is produced mainly by macrophages, and T_H2 cells.

h. Interleukin 15 (IL-15)

IL-15 stimulates NK cell proliferation and proliferation of memory T8-lymphocytes. IL-15 is produced by various cells including macrophages.

i. Interleukin-18 (IL-18)

IL-18 stimulates the production of interferon-gamma by NK cells and T-lymphocytes and thus induces cell-mediated immunity. It is produced mainly by macrophages.

A number of human cytokines produced by recombinant DNA technologies are now being used to treat various infections or immune disorders. These include:

1. recombinant interferon alfa-2a (*Roferon-A*): a cytokine used to treat Kaposi's sarcoma, chronic myelogenous leukemia, and hairy cell leukemia.
2. peginterferon alfa-2a (*Pegasys*) : used to treat hepatitis C (HCV).
3. recombinant interferon-alpha 2b (*Intron A*): a cytokine produced by recombinant DNA technology and used to treat Hepatitis B; malignant melanoma, Kaposi's sarcoma, follicular lymphoma, hairy cell leukemia, warts, and Hepatitis C.
4. peginterferon alfa-2b (*PEG-Intron; PEG-Intron Redipen*): used to treat hepatitis C (HCV).
5. recombinant Interferon alfa-2b plus the antiviral drug ribavirin (*Rebetron*): used to treat hepatitis C (HCV).
6. recombinant interferon-alpha n3 (*Alferon N*): used to treat warts.
7. recombinant iInterferon alfacon-1 (*Infergen*) : used to treat hepatitis C (HCV).
8. G-CSF (granulocyte colony stimulating factor): for reduction of infection in people after myelotoxic anticancer therapy for solid tumors.
9. GM-CSF (granulocyte-macrophage colony stimulating factor): for hematopoietic reconstruction after bone marrow transplant in people with lymphoid cancers.

TPS Question

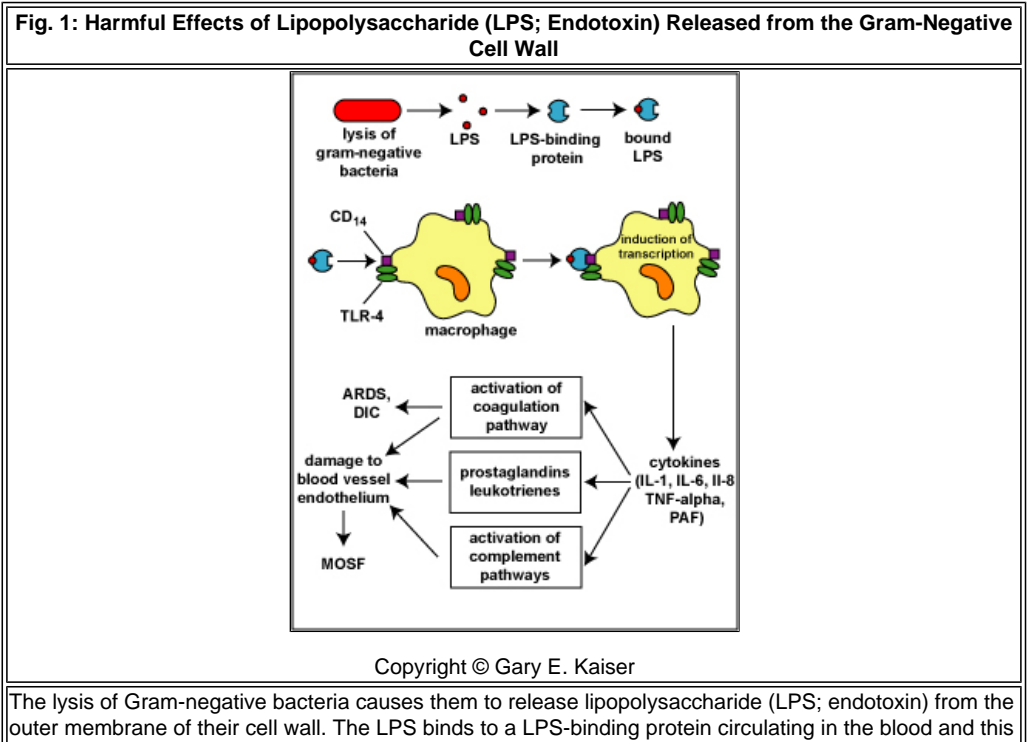
Concept Map for Cytokines Important in Innate Immunity

Quiz Group

Harmful Effects Associated with Abnormal Pattern-Recognition Receptor Responses, Variations in Innate Immune Signaling Pathways, and/or Levels of Cytokine Production

a. The Ability of Pathogen-Associated Molecular Patterns or PAMPs to Trigger the Synthesis and Secretion of Excessive Levels of Inflammatory Cytokines and Chemokines

As learned in Unit 3 under sepsis and systemic inflammatory response syndrome (SIRS), during **severe systemic infections** with large numbers of bacteria present, **high levels of cell wall PAMPs are released** resulting in **excessive cytokine production** by the defense cells and this can **harm the body (see Fig. 1)**. In addition, neutrophils start releasing their proteases and toxic oxygen radicals that kill not only the bacteria, but the surrounding tissue as well. Harmful effects include high fever, hypotension, tissue destruction, wasting, acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), and damage to the vascular endothelium. This can result in shock, multiple system organ failure (MOSF), and death.



complex, in turn, binds to a receptor molecule (CD14) found on the surface of body defense cells called macrophages. This triggers the macrophages to release various defense regulatory chemicals called cytokines, including IL-1, IL-6, IL-8, TNF-alpha, and PAF. The cytokines then bind to cytokine receptors on target cells stimulating the production of inflammatory mediators such as prostaglandins and leukotrienes as well as activating both the complement pathways and the coagulation pathway. Excessive production of clotting factors may lead to ARDS and DIC while an overproduction of prostaglandins, leukotrienes, and complement proteins can damage the vascular endothelium resulting in shock and MOSF. (LPS, lipopolysaccharide; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8, TNF-alpha, tumor necrosis factor-alpha; PAF, platelet-activating factor; ARDS, acute respiratory distress syndrome; DIC, disseminated intravascular coagulation; MOSF, multiple organ system failure.)

For more information: Review of the ability of PAMPs to trigger production of inflammatory cytokines that result in an excessive inflammatory response

b. Harmful Effects Associated with either an Overactive or an Underactive Innate Immune Response

There are a number of harmful effects that are known to occur as a result of either an overactive or an underactive innate immune response. This occurs as a result of people possessing different polymorphisms in the various genes participating in PRR signaling.

- People born with underactive PRRs or deficient PRR immune signaling pathways are at increased risk of infection by specific pathogens due to a decrease innate immune response.
- People born with overactive PRRs or deficient PRR immune signaling pathways are at increased risk of inflammatory damage by lower numbers of specific pathogens.

Examples include:

1. People with an underactive form of TLR-4, the toll-like receptor for bacterial LPS, have been found to be five times as likely to contract a severe bacterial infection over a five year period than those with normal TLR-4. People with overactive TLR-4 receptors may be more prone to developing SIRS from gram-negative bacteria.
2. Most people that die as a result of Legionnaire's disease have been found to have a mutation in the gene coding for TLR-5 that enables the body to recognize the flagella of *Legionella pneumophila*.
3. B-lymphocytes, the cells responsible for recognizing foreign antigens and producing antibodies against those antigens, normally don't make antibodies against the body's own DNA and RNA. The reason is that any B-lymphocytes that bind the body's own antigens normally undergo apoptosis, a programmed cell suicide. People with the autoimmune disease systemic lupus erythematosus have a mutation in a gene that signals the cell to undergo apoptosis. As a result, these B-cells are able to bind and engulf the body's own DNA and RNA and place them in an endosome or phagolysosome where the the DNA can be recognized by TLR-9 and the RNA by TLR-7. This, in turn, triggers those B-lymphocytes to make antibody molecules against the body's own DNA and RNA. Another gene error enables these B- cells to increase the expression of TLR-7.
4. TLR-4, MyD88, TLR-1/TLR-2 have been implicated in the production of arteriosclerosis in mice and some humans.
5. Mutations resulting in loss-of-function in the gene coding for NOD-2 that prevents the NOD-2 from recognizing muramyl dipeptide make a person more susceptible to Crohn's disease, an inflammatory disease of the large intestines. Mutations resulting in over-activation in the gene coding for NOD-2 can lead to an inflammatory disorder called Blau syndrome.
6. People with chronic sinusitis that does not respond well to treatment have decreased activity of TLR-9 and produce reduced levels of human beta-defensin 2, as well as mannan-binding lectin needed to initiate the lectin complement pathway.
7. Pathogenic strains of *Staphylococcus aureus* producing leukocidin and protein A, including MRSA, cause an increased inflammatory response. Protein A, a protein that blocks opsonization and functions as an adhesin, binds to cytokine receptors for TNF-alpha. It mimics the cytokine and induces a strong inflammatory response. As the inflammatory response attracts neutrophils to the infected area, the leukocidin causes lysis of the neutrophils. As a result, tissue is damaged and the bacteria are not phagocytosed.
8. People with chronic mucocutaneous candidiasis disease have a mutation either in the gene coding for IL-17F or the gene encoding IL-17F receptor. T_H17 cells secrete cytokines such as IL-17 that are important for innate immunity against organisms that infect mucous membranes.
9. A polymorphism in the gene for TLR-2 makes individuals less responsive to *Treponema pallidum* and *Borrelia burgdorferi* and possibly more susceptible to tuberculosis and staphylococcal infections.
10. Polymorphisms in a gene locus called *A20*, a gene that helps to control inflammation, are considered as risk alleles for rheumatoid arthritis, systemic lupus erythematosus, psoriasis, type I diabetes, and Chron's disease.
11. The innate immune response to *Mycobacterium tuberculosis* and the severity of tuberculosis depends on the response of TLR 1/2, TLR 1/6, and 9 to the bacterium. Polymorphisms in Toll-interacting protein (TOLLIP), a negative regulator of TLR signaling, influence the response of the patient to *M. tuberculosis*.

c. Therapeutic Possibilities

Researchers are now looking at various ways to either artificially activate TLRs in order to enhance immune responses or inactivate TLRs to lessen inflammatory disorders. Examples of agents being evaluated in clinical studies or animal studies include:

1. TLR activators to activate immune responses

- a. Both TLR-4 and TLR-9 activators are being tried in early clinical trials as vaccine adjuvants to improve the immune response to vaccines. TLR-9 activators are being tried as an adjuvant for the hepatitis B and anthrax vaccines and a TLR-4 activator is being tried as an adjuvant for the vaccine against the human papillomaviruses that cause most cervical cancer.
- b. Both TLR-7 and TLR-9 activators are being tried in early clinical trials as an antiviral against hepatitis C. Activation of these TLRs triggers the synthesis and secretion of type I interferons that block viral replication within infected host cells.
- c. TLR-9 activators are being tried in early clinical trials as an adjuvant for chemotherapy in the treatment of lung cancer.
- d. TLR-9 activators are being tried in early clinical trials to help in the treatment and prevention of allergies and asthma. Activation of TLR-9 in macrophages and other cells stimulates these cells to kill TH2 cells, the subclass of T-helper lymphocytes responsible for most allergies and asthma.

2. TLR inhibitors to suppress immune responses

- a. General TLR inhibitors might one day be used to treat autoimmune disorders.
- b. A TLR-4 inhibitor, a mimic of the endotoxin from the gram-negative cell wall, is being tried in early clinical trials to block or reduce the death rate from Gram-negative sepsis and SIRS.
- c. TLR-4, TLR-2, and MyD88 inhibitors might possibly one day lessen atherosclerotic plaques and the risk of heart disease.

Of course using TLR activators or TLR inhibitors to turn up or turn down immune responses also carries risks. Trying to suppress harmful inflammatory responses may also result in increased susceptibility to infections; trying to activate immune responses could lead to SIRS or autoimmune disease.

For more information: Review of pattern-recognition receptors (PRRs)

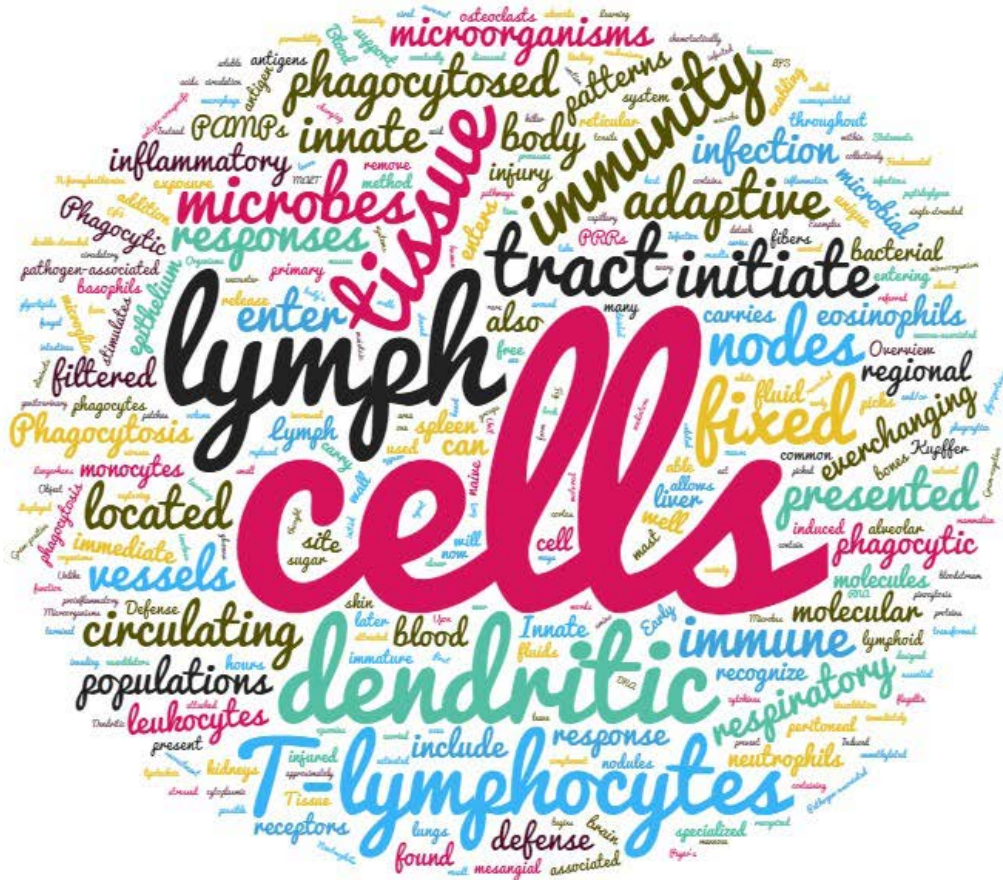


Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

Innate Immunity

Early Induced Innate Immunity: An Overview of Phagocytic Defense



Fundamental Statement for this Softchalk Lesson:

1. Phagocytosis is the primary method used by the body to remove free microorganisms in the blood and tissue fluids.
2. An inflammatory response to injury and/or infection allows phagocytes to leave the bloodstream, enter the tissue, and go to the site of infection or injury.
3. Microorganisms entering lymph nodules found in the respiratory, gastrointestinal, and genitourinary tract can be phagocytosed by fixed macrophages and dendritic cells and presented to B-lymphocytes and T-lymphocytes to initiate adaptive immune responses.
4. Tissue fluid picks up microbes in the tissue, enters the lymph vessels as lymph, and carries the microbes to regional lymph nodes where they are filtered out and phagocytosed by fixed macrophages and dendritic cells and presented to the circulating B-lymphocytes and T-lymphocytes to initiate adaptive immune responses.
5. Dendritic cells located throughout the epithelium of the skin, the respiratory tract, and the gastrointestinal tract phagocytose microbes, enter lymph vessels, and carry the microbes to regional lymph nodes where the dendritic cells present antigens associated with the microbes to the ever changing populations of naive T-lymphocytes.
6. Blood carries microorganisms to the spleen where they are filtered out and phagocytosed by fixed macrophages and dendritic cells and presented to the circulating B-lymphocytes and T-lymphocytes to initiate adaptive immune responses.
7. There are also specialized macrophages and dendritic cells located in the brain (microglia), lungs (alveolar macrophages), liver (Kupffer cells), kidneys (mesangial cells), bones (osteoclasts), and the gastrointestinal tract (peritoneal macrophages).

Detailed Learning Objectives

1*. Briefly describe the role of the following as they relate to phagocytosis:

- a. inflammation
- b. lymph nodules
- c. lymph nodes
- d. spleen

(*) = Common theme throughout the course

Early Induced Innate Immunity: An Overview of Phagocytic Defense

Phagocytosis is the primary method used by the body to remove free microorganisms in the blood and tissue fluids

. Phagocytic cells include neutrophils, eosinophils, monocytes, macrophages, dendritic cells, and B-lymphocytes.

The body's phagocytic cells are able to encounter these microorganisms in a variety of ways:

a. **Infection or tissue injury** stimulates mast cells, basophils, and other cells to release vasodilators to initiate the **inflammatory response**. Vasodilation results in increased capillary permeability, enabling phagocytic white blood cells such as neutrophils, monocytes, and eosinophils - as well as other leukocytes - to enter the tissue around the injured site. The leukocytes are then chemotactically attracted to the area of infection. In other words, **inflammation allows phagocytes to enter the tissue and go to the site of infection**. Neutrophils are the first to appear and are later replaced by macrophage.

For more information: Review of leukocytes

For more information: Review of macrophages and dendritic cells

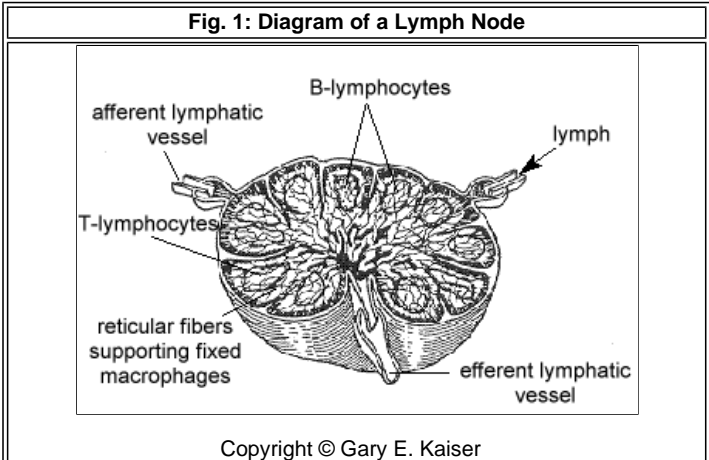
b. **Lymph nodules** are unencapsulated masses of lymphoid tissue containing fixed macrophages and ever changing populations of B-lymphocytes and T-lymphocytes. They are located in the respiratory tract, the liver, and the gastrointestinal tract and are collectively referred to as **mucosa-associated lymphoid tissue** or MALT. Examples include the adenoids and tonsils in the respiratory tract and the Peyer's patches on the small intestines. Organisms entering these systems can be **phagocytosed by fixed macrophages and dendritic cells** and presented to B-lymphocytes and T-lymphocytes to initiate adaptive immune responses.

For more information: Preview of B-lymphocytes

For more information: Preview of T4-lymphocytes

For more information: Preview of T8-lymphocytes

c. **Tissue fluid** picks up microbes and then enters the lymph vessels as lymph. Lymph vessels carry the lymph to regional **lymph nodes (see Fig. 1)**. Lymph nodes contain many reticular fibers that support fixed macrophages and dendritic cells as well as ever changing populations of circulating B-lymphocytes and T-lymphocytes. Microbes picked up by the lymph vessels are **filtered out and phagocytosed in the lymph nodes by these fixed macrophages and dendritic cells** and presented to the circulating B-lymphocytes and T-lymphocytes to initiate adaptive immune responses. The lymph eventually enters the circulatory system at the heart to maintain the fluid volume of the circulation.



d. In addition, Langerhans' cells - immature dendritic cells - are located throughout the epithelium of the skin, the respiratory tract, and the gastrointestinal tract where in their immature form they are attached by long cytoplasmic processes. Upon capturing antigens through pinocytosis and phagocytosis and becoming activated by proinflammatory cytokines, the dendritic cells detach from the epithelium, enter lymph vessels, and are carried to regional lymph nodes. By the time they enter the lymph nodes, they have matured and are now able to present antigen to the ever changing populations of naive T-lymphocytes located in the cortex of the lymph nodes.

e. The spleen contains many reticular fibers that support fixed macrophages and dendritic cells, as well as ever changing populations of circulating B-lymphocytes and T-lymphocytes. Blood carries microorganisms to the **spleen** where they are **filtered out and phagocytosed by the fixed macrophages and dendritic cells** and presented to the circulating B-lymphocytes and T-lymphocytes to initiate adaptive immune responses.

To view a diagram of the lymphatics system, see the Innerbody.com Webpage.

f. There are also specialized macrophages and dendritic cells located in the brain (microglia), lungs (alveolar macrophages), liver (Kupffer cells), kidneys (mesangial cells), bones (osteoclasts), and the gastrointestinal tract (peritoneal macrophages).

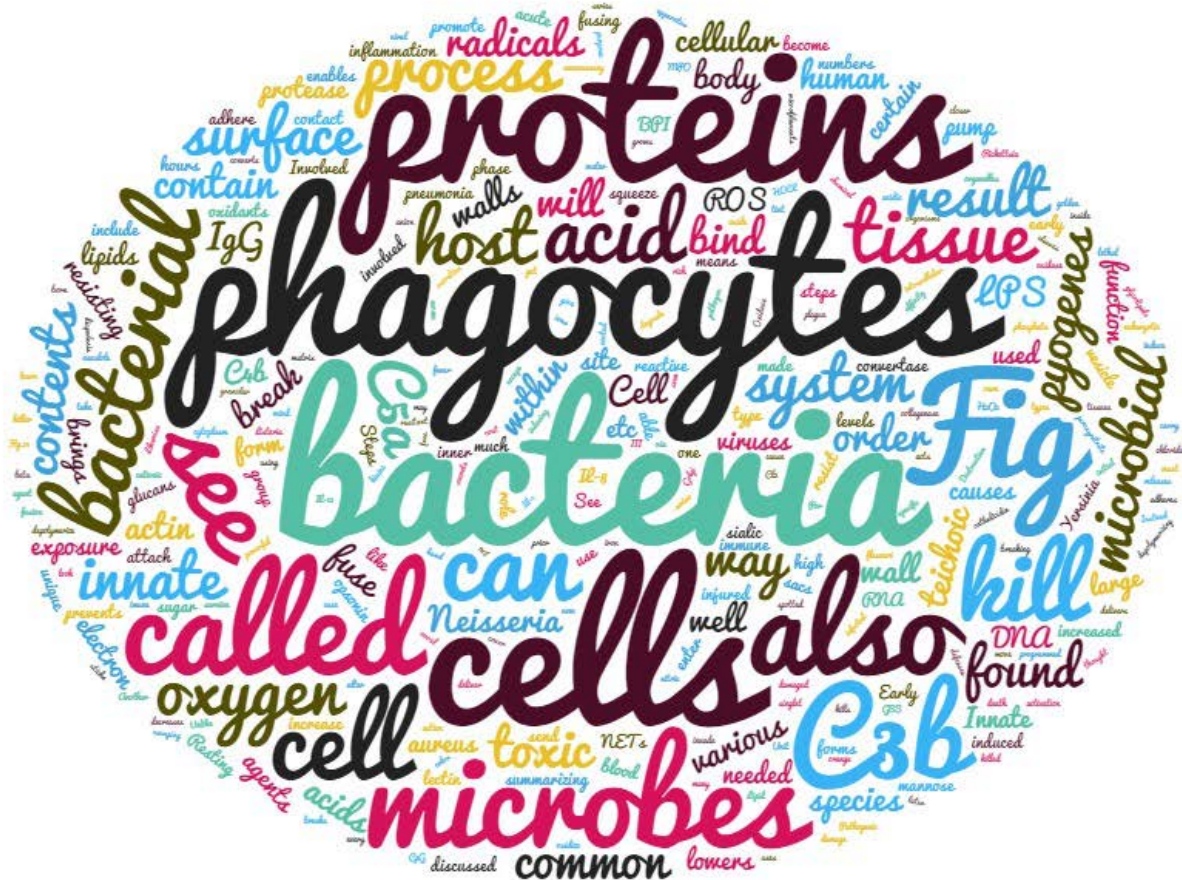


Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

Innate Immunity

Early Induced Innate Immunity: The Steps Involved in Phagocytosis



1. Resting phagocytes are activated by inflammatory mediators and produce surface receptors that increase their ability to adhere to the inner surface of capillary walls enabling them to squeeze out of the capillary and enter the tissue, a process called diapedesis.
2. Activation also enables phagocytes to produce endocytic pattern-recognition receptors that recognize and bind to microbial PAMPs in order to attach the microbe to the phagocyte, as well as to exhibit increased metabolic and microbicidal activity.
3. Phagocytes then use chemotaxis to move towards an increasing concentration of some attractant such as bacterial factors or defense molecules.
4. Attachment of phagocytes to the microbes or cells can be through unenhanced attachment or enhanced attachment.
5. Unenhanced attachment is the recognition of pathogen-associated molecular patterns or PAMPs by endocytic pattern-recognition receptors on the surface of the phagocytes.
6. Enhanced attachment, or opsonization, is the attachment of microbes to phagocytes by way of an antibody molecule called IgG, the complement proteins C3b and C4b, and acute phase proteins such as mannose-binding lectin (MBL) and C-reactive protein (CRP).
7. Following attachment, polymerization and then depolymerization of actin filaments send pseudopods out to engulf the microbe and place it in an endocytic vesicle called a phagosome.
8. During this process, an electron pump brings protons (H^+) into the phagosome to lower the pH within the phagosome to a pH that is correct for the acid hydrolases to effectively break down cellular proteins.
9. Phagocytes contain membranous sacs called lysosomes that contain various digestive enzymes, microbicidal chemicals, and toxic oxygen radicals. The lysosomes fuse with the phagosomes containing the ingested microbes and the microbes are destroyed.
10. If the infection site contains very large numbers of microorganisms and high levels of inflammatory cytokines and chemokines are being produced in response to PAMPs, the phagocyte will empty the contents of its lysosomes in order to kill the microorganisms or cell extracellularly.
11. Lysosomal contents released during extracellular killing also kill surrounding host cells and tissue. Most tissue destruction associated with

infections is a result of extracellular killing by phagocytes.

Common Course Objective

- 1. Describe the stages of phagocytosis

Detailed Learning Objectives

- 1**. Describe the following steps in phagocytosis:
 - a. activation
 - b. chemotaxis
 - c. attachment (both unenhanced and enhanced)
 - d. ingestion
 - e. destruction
- 2*. State what happens when either phagocytes are overwhelmed with microbes or they adhere to cells too large to be phagocytosed.
- 3*. Describe what causes most of the tissue destruction seen during microbial infections.
- 4*. Compare the oxygen-dependent and oxygen-independent killing systems of neutrophils and macrophages.
- 5*. Briefly describe the role of autophagy in removing intracellular microbes.

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Questions

Early Induced Innate Immunity: The Steps Involved in Phagocytosis

There are a number of distinct steps involved in phagocytosis:

1. Activation of the Phagocyte

Resting phagocytes are activated by inflammatory mediators such as bacterial products (bacterial proteins, capsules, LPS, peptidoglycan, teichoic acids, etc.), complement proteins, inflammatory cytokines, and prostaglandins. As a result, the circulating phagocytes produce surface glycoprotein receptors that increase their ability to **adhere to the inner surface of capillary walls, enabling them to squeeze out of the capillary and be attracted to the site of infection.**

In addition, they **produce endocytic pattern-recognition receptors** that **recognize and bind to pathogen-associated molecular patterns** or PAMPs - components of common microbial molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, and mannose-rich glycans that are not found in human cells - to attach the microbe to the phagocyte for what is called unenhanced attachment (discussed below).

For more information: Review of pathogen-associated molecular patterns (PAMPs)

For more information: Review of pattern-recognition receptors (PRRs)

For more information: Review of leukocytes

They also exhibit **increased metabolic and microbicidal activity** by increasing their production of ATPs, lysosomal enzymes, lethal oxidants, etc.

2. Chemotaxis of Phagocytes (for wandering macrophages, neutrophils, and eosinophils)

Chemotaxis is the **movement of phagocytes toward an increasing concentration of some attractant** such as bacterial factors (bacterial proteins, capsules, LPS, peptidoglycan, teichoic acids, etc.), complement proteins (C5a), chemokines (chemotactic cytokines such as interleukin-8 secreted by various cells), fibrin split products, kinins, and phospholipids released by injured host cells.

Flash animation showing the role of C5a in vasodilation, the chemotaxis of phagocytes towards C5a, and their attachment to the opsonin C3b as a result of the complement pathways.

Copyright © Gary E. Kaiser
html5 version of animation for iPad showing the role of C5a in vasodilation, the chemotaxis of phagocytes towards C5a, and their attachment to the opsonin C3b as a result of the complement pathways.
During the complement pathways, complement proteins such as C3a, C3b, C4a, C4b, and C5a are produced. These all play a role in inflammation and phagocytosis. C5a, C3a, and C4a stimulate mast cells to release histamine and other vasoactive agents to promote inflammation and diapedesis. C5a also functions as a chemoattractant for phagocytes. Most C3b and C4b binds to antigens on the microbial surface. The phagocytes are then able to bind to the C3b attached to the surface of the microorganism allowing for opsonization (enhanced attachment).

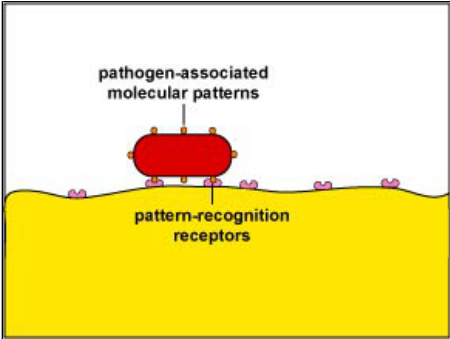
Movie showing chemotaxis of neutrophils
Chemotaxis of Neutrophils. © From Intimate Strangers: Unseen Life on Earth. Created by Mondo Media. Peter Baker, Executive Producer. Licensed for use, ASM MicrobeLibrary.

As learned under Bacterial Pathogenesis, **some microbes**, such as the influenza A viruses , *Mycobacterium tuberculosis*, blood invasive strains of *Neisseria gonorrhoeae*, and *Bordetella pertussis* **have been shown to block chemotaxis**.

3. Attachment of the Phagocyte to the Microbe or Cell

Attachment of microorganisms is necessary for ingestion. Attachment may be unenhanced or enhanced.

a. **Unenhanced attachment**
Unenhanced attachment is the innate recognition of **pathogen-associated molecular patterns or PAMPs** - components of common molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, and glucans common in microbial cell walls but not found on human cells - by means of **endocytic pattern-recognition receptors**, such as scavenger receptors and mannose receptors, on the surface of the phagocytes (see Fig. 1).

Fig. 1: Unenhanced Attachment of Bacteria to Phagocytes

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Glycoprotein molecules known as pattern-recognition receptors are found on the surface of phagocytes. They are so named because they recognize and bind to pathogen-associated molecular patterns - components of common molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, and glucans - found in many microorganisms.

Flash animation illustrating the function of endocytic pattern-recognition receptors on phagocytes.
Copyright © Gary E. Kaiser
html5 version of animation for iPad illustrating the function of endocytic pattern-recognition receptors on phagocytes.
Glycoprotein molecules known as endocytic pattern-recognition receptors are found on the surface of phagocytes. They are so named because they recognize and bind to pathogen-associated molecular patterns - molecular components associated with microorganisms but not found as a part of eukaryotic cells. These include bacterial molecules such as peptidoglycan, lipoteichoic acids, mannans, and lipopolysaccharide (LPS). These receptors enable the phagocyte to attach to the cell wall of the microorganism so it can be engulfed and destroyed by lysosomes.

For more information: Review of pathogen-associated molecular patterns (PAMPs)
For more information: Review of pattern-recognition receptors (PRRs)

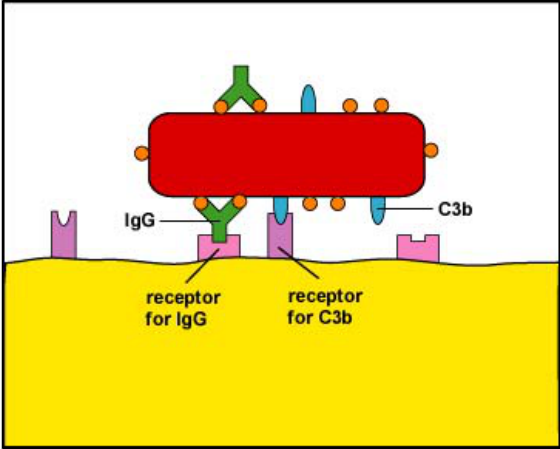
Movie of a bacterium being engulfed by a neutrophil.

Phagocytosis. © James Sullivan, author. Licensed for use, ASM MicrobeLibrary.

b. Enhanced attachment

Enhanced attachment is the attachment of microbes to phagocytes by way of an antibody molecule called IgG, the complement proteins C3b and C4b produced during the complement pathways (see Fig. 2), and acute phase proteins such as mannose-binding lectin (MBL) and C-reactive protein (CRP). Molecules such as IgG, C3b, and mannose-binding lectin (MBL) that promote enhanced attachment are called **opsonins** and the process is also known as **opsonization**. Enhanced attachment is much more specific and efficient than unenhanced.

Fig. 2: Enhanced Attachment of Bacteria to Phagocytes



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One of the functions of certain antibody molecules known as IgG is to stick antigens such as bacterial proteins and polysaccharides to phagocytes. The tips of the antibody, the Fab portion, have a shape that fits epitopes, portions of an antigen with a complementary shape. The stalk of the antibody is called the Fc portion and is able to bind to Fc receptors on phagocytes. Also, when body defense pathways known as the complement pathways are activated, one of the beneficial defense proteins made is called C3b. C3b binds by one end to bacterial surface proteins and by the other end to C3b receptors on phagocytes. The IgG and C3b are also known as opsonins and the process of enhanced attachment is also called opsonization.

Flash animation illustrating the function of enhanced attachment by way of IgG.

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html5 version of animation for iPad illustrating the function of enhanced attachment by way of IgG.

The Fab portion of IgG binds to epitopes of a microbe. The Fc portion can now attach the microbe to Fc receptors on phagocytes for enhanced attachment, also known as opsonization. Once attached to the phagocyte by way of IgG, the microbe can be engulfed more efficiently and placed in a phagosome, and destroyed by lysosomes.

Flash animation showing the role of C5a in vasodilation, the chemotaxis of phagocytes towards C5a, and their attachment to the opsonin C3b as a result of the complement pathways.

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html5 version of animation for iPad showing the role of C5a in vasodilation, the chemotaxis of phagocytes towards C5a, and their attachment to the opsonin C3b as a result of the complement pathways.

During the complement pathways, complement proteins such as C3a, C3b, C4a, C4b, and C5a are produced. These all play a role in inflammation and phagocytosis. C5a, C3a, and C4a stimulate mast cells to release histamine and other vasoactive agents to promote inflammation and diapedesis. C5a also functions as a chemoattractant for phagocytes. Most C3b and C4b binds to antigens on the microbial surface. The phagocytes are then able to bind to the C3b attached to the surface of the microorganism allowing for opsonization (enhanced attachment).

For more information: Preview of antibodies

For more information: Review of the complement pathways

c. Extracellular trapping with NETs

In response to certain pathogen associated molecular patterns such as LPS, and certain cytokines such as IL-8, neutrophils release DNA and antimicrobial granular proteins. These neutrophil extracellular traps (NETs) bind to bacteria, prevent them from spreading, and kill them with antimicrobial proteins (see Fig. 3 and Fig. 4).

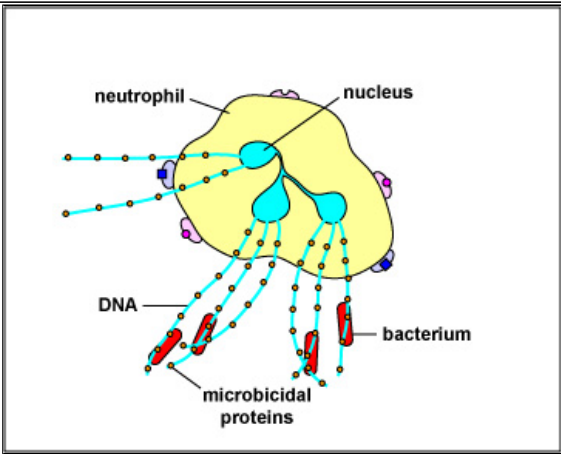
Fig. 3: Neutrophil NETS Trapping *Salmonella typhimurium*



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V. Brinkmann, U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann,
D.S. Weiss, Y. Weinrauch, and A. Zychlinsky, *Science* 303, 1534 (2004).
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Scanning electron micrograph of *Salmonella typhimurium* trapped by neutrophil extracellular traps (NETs) with antibacterial proteins. As a part of innate immunity, neutrophils release chromatin with granular proteins that trap bacteria, prevent them from spreading, and kill them with antimicrobial proteins.

Fig. 4: Neutrophil NETS Trapping and Killing Bacteria



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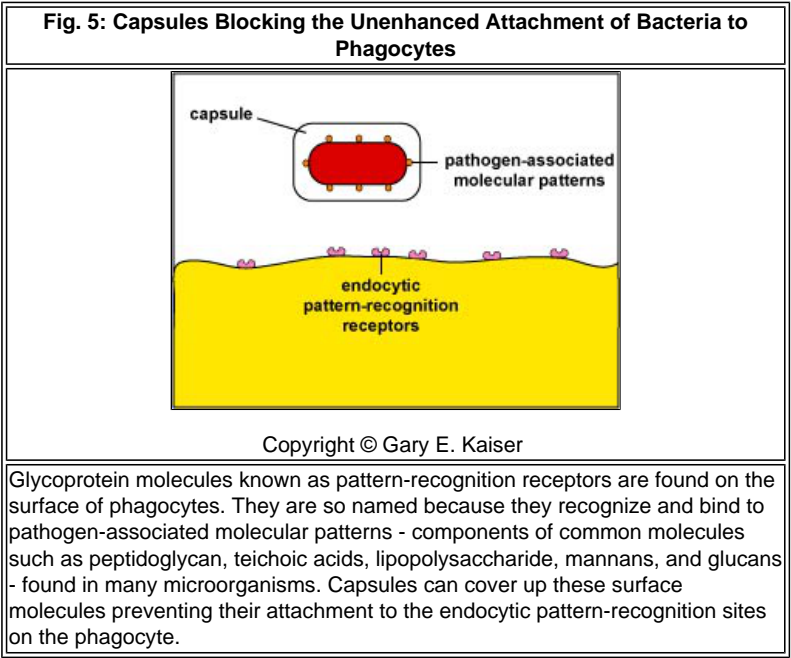
In response to certain pathogen associated molecular patterns such as LPS, and certain cytokines such as IL-8, neutrophils release DNA and antimicrobial granular proteins. These neutrophil extracellular traps (NETs) bind to bacteria, prevent them from spreading, and kill them with antimicrobial proteins such as histones and elastins. One hypothesis, shown in this animation, proposes that the NETs are produced by living neutrophils in response to bacteria. Alternately, NETs may be released as a result of necrotic cell death of neutrophils.

GIF animation showing the formation of neutrophil NETs

Quiz Group

As learned under Bacterial Pathogenecity, **some microorganisms are more resistant to phagocytic attachment.**

- a. **Capsules can resist unenhanced attachment** by preventing the endocytic pattern recognition receptors on phagocytes from recognizing the bacterial cell wall components and mannose-containing carbohydrates (**see Fig. 5**). *Streptococcus. pneumonia* activates the classical complement pathway, but resists C3b opsonization, and complement causes further inflammation in the lungs.



Flash animation illustrating how capsules can block unenhanced attachment of pathogen-associated molecular patterns to endocytic pattern-recognition receptors on phagocytes.

Copyright © Gary E. Kaiser

html5 version of animation for iPad illustrating how capsules can block unenhanced attachment of pathogen-associated molecular patterns to endocytic pattern-recognition receptors on phagocytes.

Glycoprotein molecules known as endocytic pattern-recognition receptors are found on the surface of phagocytes. They are so named because they recognize and bind to pathogen-associated molecular patterns - molecular components associated with microorganisms but not found as a part of eukaryotic cells. These include bacterial molecules such as peptidoglycan, lipoteichoic acids, and lipopolysaccharide (LPS). These receptors enable the phagocyte to attach to the cell wall of the microorganism so it can be engulfed and destroyed by lysosomes. Capsules can cover the pathogen-associated molecular patterns blocking their binding to endocytic pattern-recognition receptors.

Movie of an encapsulated bacterium resisting phagocytosis by a neutrophil

Phagocytosis. © James Sullivan, author. Licensed for use, ASM MicrobeLibrary.

- b. **Some capsules prevent the formation of C3 convertase**, an early enzyme in the complement pathways. Without this enzyme, **the opsonins C3b and C4b**, as well as the other beneficial proteins are not produced.

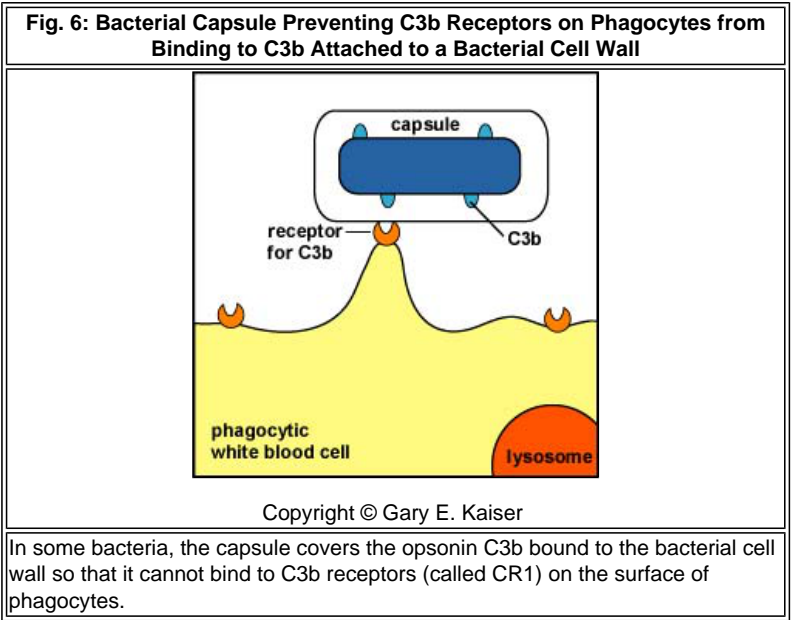
Flash animation showing an encapsulated bacterium resisting phagocytosis by blocking C3b.

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html5 version of animation for iPad showing an encapsulated bacterium resisting phagocytosis by blocking C3b.

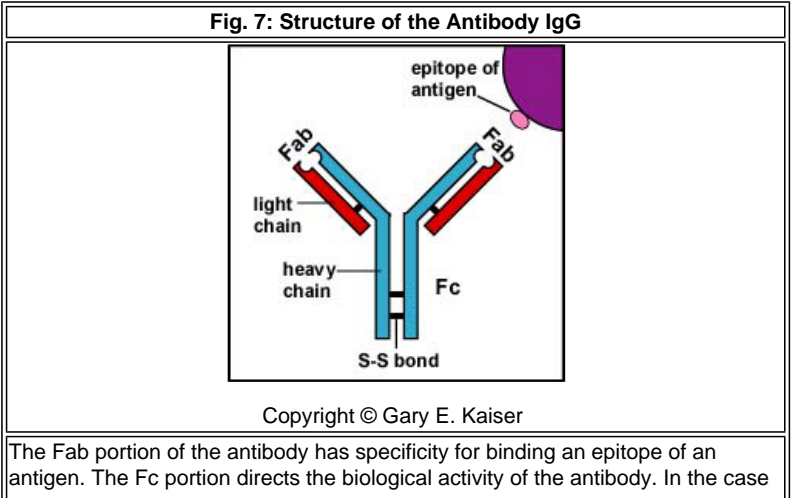
In some bacteria, the capsule covers the opsonin C3b bound to the bacterial cell wall so that it can't bind to C3b receptors (called CR1) on the surface of phagocytes.

- c. **Other capsules, rich in sialic acid, a common component of host cell glycoprotein, have an affinity for serum protein H**, a complement regulatory protein that leads to the degradation of the opsonin C3b by factor I and the formation of C3 convertase. (Serum protein H is what normally leads to the degradation of any C3b that binds to host glycoproteins so that we don't stick our own phagocytes to our own cells with C3b.)
- d. **Some capsules simply cover the C3b** that does bind to the bacterial surface and **prevent the C3b receptor on phagocytes from making contact with the C3b** (see Fig. 6). This is seen with the capsule of *Streptococcus pneumoniae*.



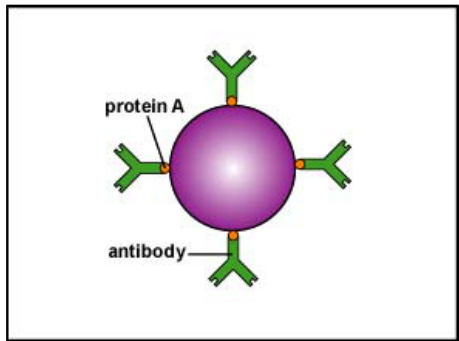
Flash animation showing an encapsulated bacterium resisting phagocytosis by blocking C3b.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing an encapsulated bacterium resisting phagocytosis by blocking C3b.
In some bacteria, the capsule covers the opsonin C3b bound to the bacterial cell wall so that it can't bind to C3b receptors (called CR1) on the surface of phagocytes.

- e. *Neisseria meningitidis* has a **capsule composed of sialic acid** while *Streptococcus pyogenes* (group A beta streptococci) has a **capsule made of hyaluronic acid**. Both of these polysaccharides closely resemble carbohydrates found in human tissue polysaccharides and because they are not recognized as foreign by the lymphocytes that carry out the immune responses, **antibodies are not made against these capsules**. Likewise, some bacteria are able to **coat themselves with host proteins** such as fibronectin, lactoferrin, or transferrin and in this way avoid antibodies.
- f. An outer membrane molecule of *Neisseria gonorrhoeae* called Protein II and the M-protein of *Streptococcus pyogenes* allow these bacteria to be more resistant to phagocytic engulfment. The **M-protein** of *S. pyogenes*, for example, **binds factor H of the complement pathway and this results in the degradation of the opsonin C3b by factor I** and the formation of C3 convertase. *S. pyogenes* also produces a protease that **cleaves the complement protein C5a**.
- g. *Staphylococcus aureus* produces **protein A** while *Streptococcus pyogenes* produces **protein G**. Both of these proteins **bind to the Fc portion of antibodies** (see Fig. 7) and in this way the bacteria become coated with antibodies in a way that **does not result in opsonization** (see Fig. 8).



of IgG, the Fc portion can bind to phagocytes for enhanced attachment (opsonization) as well as activate the classical complement pathway. Antibodies are composed of 4 protein chains: 2 identical heavy chains and 2 identical light chains. Disulfide (S-S) bonds join the protein chains together

Fig. 8: *Staphylococcus aureus* Resisting Opsonization via Protein A



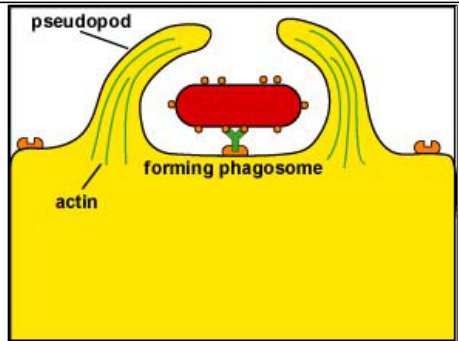
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The Fc portion of the antibody IgG, the portion that would normally binds to Fc receptors on phagocytes, instead binds to protein A on *Staphylococcus aureus*. In this way the bacterium becomes coated with a protective coat of antibodies that do not allow for opsonization.

4. Ingestion of the Microbe or Cell by the Phagocyte

Following attachment, polymerization and then depolymerization of actin filaments send pseudopods out to engulf the microbe (see Fig. 9) and place it in an endocytic vesicle called a phagosome (see Fig. 10).

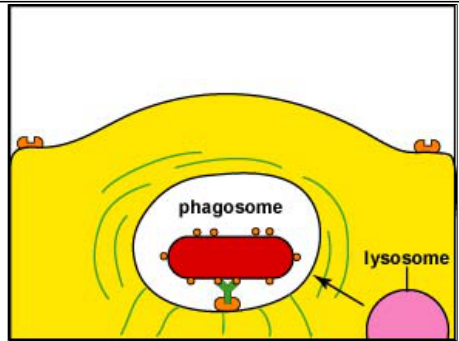
Fig. 9: Formation of Pseudopods by Rearrangement of Actin Molecules



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Following attachment, polymerization and depolymerization of actin molecules send pseudopods out to engulf the bacterium and place it in a vesicle called a phagosome.

Fig. 10: Placing the Bacterium in a Phagosome



Copyright © Gary E. Kaiser
Following engulfment, the bacterium is placed in a vesicle called a phagosome.

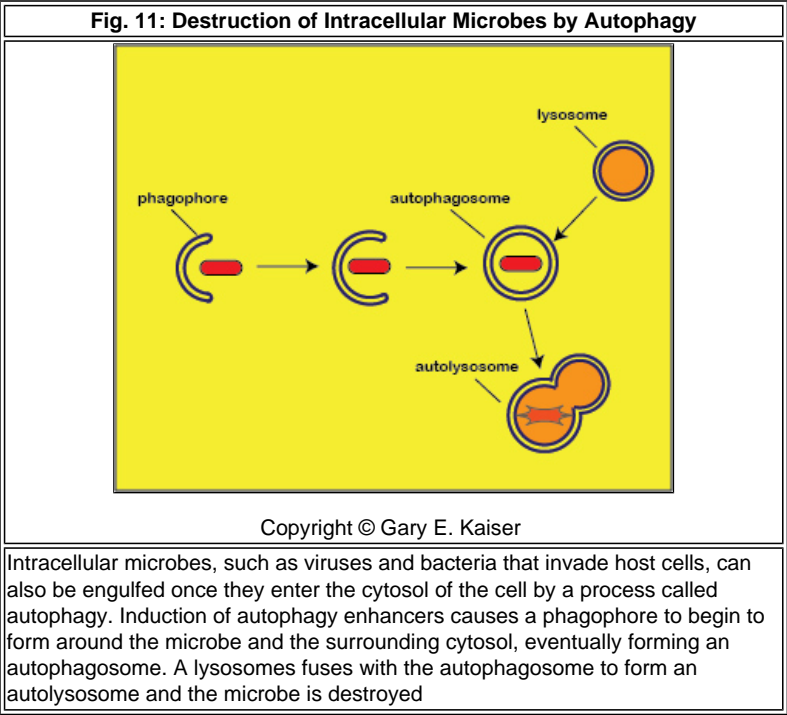
Flash animation showing ingestion and phagosome formation.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing ingestion and phagosome formation.
Following attachment, polymerization and then depolymerization of actin filaments send pseudopods out to engulf the microbe and place it in a vesicle called a phagosome.

During this process, **an electron pump brings protons (H⁺) into the phagosome**. This **lowers the pH within the phagosome** to 3.5 - 4.0 so that when a lysosome fuses with the phagosome, the pH is correct for the acid hydrolases to effectively **break down cellular proteins**. The acidification also releases defensins, cathelicidin, and bacterial permeability inducing protein (BPI), peptides and enzymes that can kill microbes, from a matrix and enabling their activation.

Flash animation showing acidification of the phagosome following ingestion.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing acidification of the phagosome following ingestion.
During phagosome formation, an electron pump brings protons (H ⁺) into the phagosome. This lowers the pH within the phagosome so that when a lysosome fuses with the phagosome, the pH is correct for the acid hydrolases to effectively break down cellular proteins.

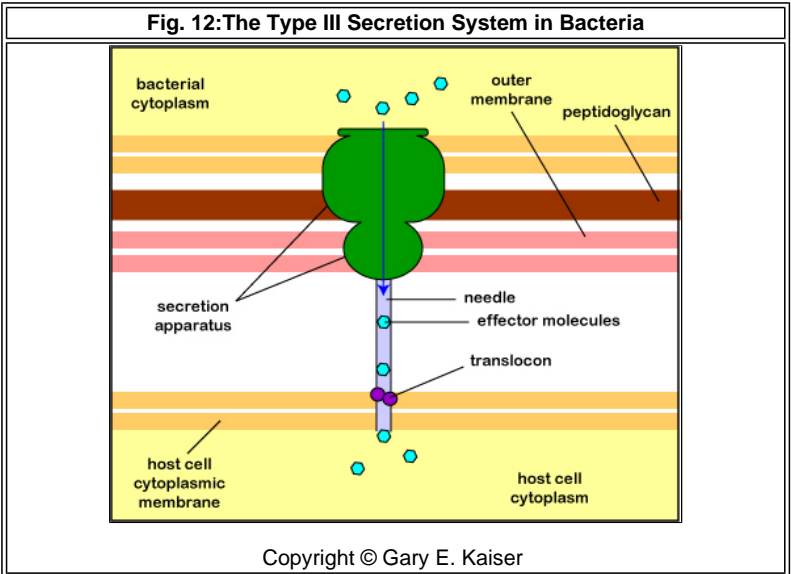
View a scanning electron micrographs of a macrophage with pseudopods and a macrophage phagocytosing *E. coli* on a blood vessel; courtesy of Dennis Kunkel's Microscopy.

Intracellular microbes, such as viruses and bacteria that invade host cells, can also be engulfed once they enter the cytosol of the cell by a process called autophagy. A membrane-bound compartment called an autophagosome grows around the microbe and the surrounding cytosol and subsequently delivers it to lysosomes for destruction (**see Fig. 11**). (This process is also used by eukaryotic cells to engulf and degrade unnecessary or dysfunctional cellular components such as damaged organelles.)

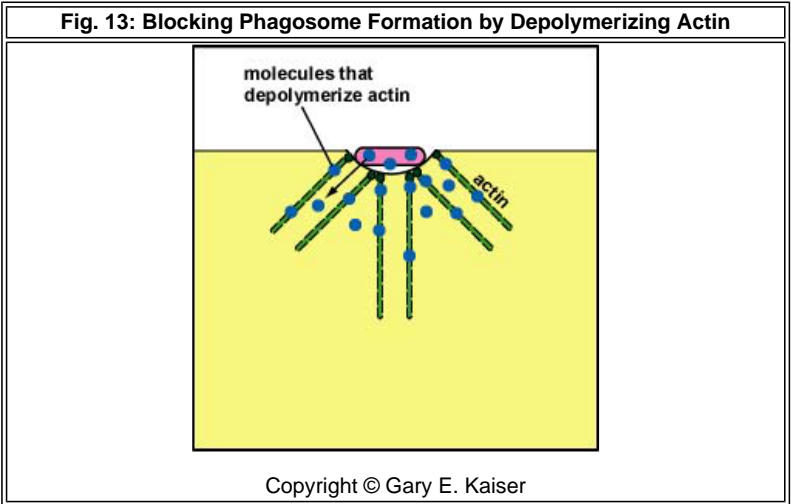


As learned under Bacterial Pathogenesis, **some microorganisms are more resistant to phagocytic ingestion.**

a. Pathogenic *Yersinia*, such as *Yersinia pestis*, contact phagocytes and, by means of a type III secretion system (see Fig. 12), deliver proteins which depolymerize the actin microfilaments needed for phagocytic engulfment into the phagocytes (see Fig. 13). Another *Yersinia* protein degrades C3b and C5a.



Many bacteria involved in infection have the ability to co-opt the functions of the host cell for the bacterium's own benefit. This is done by way of bacterial secretions systems that enable the bacterium to directly inject bacterial effector molecules into the cytoplasm of the host cell in order to alter its cellular machinery or cellular communication. The most common type is the type 3 secretion system. A secretion apparatus in the cytoplasmic membrane and cell wall of the bacterium polymerizes a hollow needle that is lowered to the cytoplasmic membrane of the host cell and a translocon protein is then delivered to anchor the needle to the host cell. Effector proteins in the bacterium can now be injected into the cytoplasm of the host cell. The delivery system is sometimes called an injectosome.



Molecules of some bacteria, through a type III secretion system, deliver proteins which depolymerize the actin microfilaments of the phagocyte used for phagocytic engulfment.

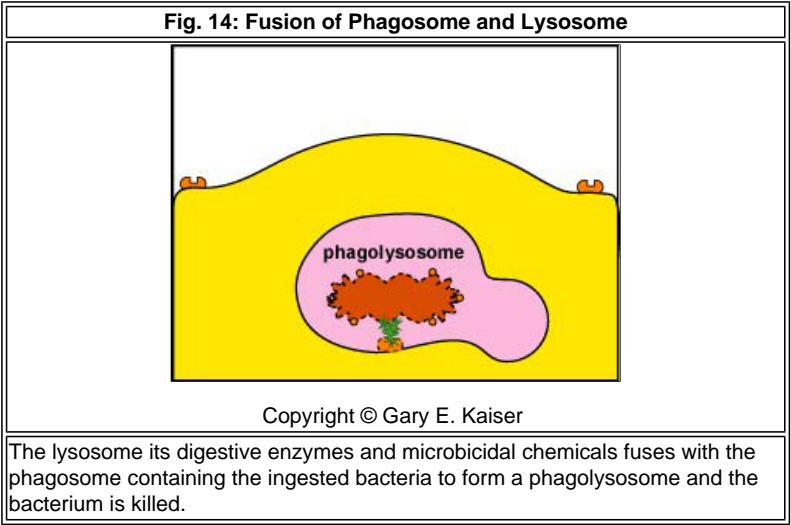
GIF animation showing bacteria depolymerizing actin in order to resist phagocytosis

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b. Some bacteria, like *Mycobacterium tuberculosis*, *Salmonella*, and *Listeria monocytogenes* can **block autophagy.**

5. Destruction of the Microbe or Cell

Phagocytes contain membranous sacs called **lysosomes** produced by the Golgi apparatus that **contain various digestive enzymes, microbicidal chemicals, and toxic oxygen radicals**. The **lysosomes** travel along microtubules within the phagocyte and fuse with the phagosomes containing the ingested microbes and the microbes are destroyed (**see Fig. 14**).



Flash animation showing intracellular destruction.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing intracellular destruction.
Lysosomes move along the cytoskeleton and fuse with phagosomes to form phagolysosomes.

<p>3D animation illustrating organelles moving along a microtubule.</p> <p>From Graham Johnson, Fifth Element. This animation takes some time to download.</p>

Flash animation summarizing phagocytosis by unenhanced attachment.	
Copyright © Gary E. Kaiser	
html5 version of animation for iPad summarizing phagocytosis by unenhanced attachment.	
Unenhanced attachment is a general recognition of what are called pathogen-associated molecular patterns or PAMPs- components of common molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, and glucans common in microbial cell walls but not found on human cells - by means of glycoproteins known as endocytic pattern-recognition receptors on the surface of the phagocytes. Following attachment, polymerization and then depolymerization of actin filaments send pseudopods out to engulf the microbe and place it in a vesicle called a phagosome. Finally, lysosomes, containing digestive enzymes and microbicidal chemicals, fuse with the phagosome containing the ingested microbe and the microbe is destroyed.	

Flash animation summarizing phagocytosis by enhanced attachment (opsonization).
Copyright © Gary E. Kaiser
html5 version of animation for iPad summarizing phagocytosis by enhanced attachment (opsonization).
Enhanced attachment is the attachment of microbes to phagocytes by way of molecules such as the antibody molecule IgG or proteins produced during the complement pathways called C3b and C4b. Following attachment, polymerization and then depolymerization of actin filaments send pseudopods out to engulf the microbe and place it in a vesicle called a phagosome. Finally, lysosomes, containing digestive enzymes and microbicidal chemicals, fuse with the phagosome containing the ingested microbe and the microbe is destroyed.

As learned under bacterial pathogenesis, **some bacteria are more resistant to phagocytic destruction once engulfed.**

- a. Some bacteria, such as *Legionella pneumophila* and *Mycobacterium* species, **cause the phagocytic cell to place them into an endocytic vacuole via a pathway that decreases their exposure to toxic oxygen compounds.**
- b. Some bacteria, such as *Salmonella*, are **more resistant to toxic forms of oxygen and to defensins** (toxic peptides that kill bacteria).
- c. Some bacteria, such as *Shigella flexneri* and the spotted fever *Rickettsia*, **escape from the phagosome into the cytoplasm prior to the phagosome fusing with a lysosome.**

Flash animation showing a bacterium resisting phagocytosis by escaping from a phagosome prior to the phagosome fusing with the lysosome.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a bacterium resisting phagocytosis by escaping from a phagosome prior to the phagosome fusing with the lysosome.
Certain bacteria can escape from the phagosome into the cytoplasm before the phagosome is able to fuse with the lysosome. As a result, the ingested bacteria are not killed.

- d. *Neisseria gonorrhoeae* produces Por protein (protein I) that prevents phagosomes from fusing with lysosomes enabling the bacteria to survive inside phagocytes.

Flash animation showing a bacterium resisting phagocytosis by blocking the fusion of the phagosome with the lysosome.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a bacterium resisting phagocytosis by blocking the fusion of the phagosome with the lysosome.
Certain bacteria alter the phagosomal membrane, prevent the phagosome from fusing with the lysosome. Since the lysosome does not fuse with the phagosome, the ingested bacteria are not killed.

- e. Some bacteria, such as species of *Salmonella*, *Mycobacterium*, *Legionella*, and *Chlamydia*, **block the vesicular transport machinery that enables the phagosome to fuse with the lysosome.**

Flash animation showing a bacterium resisting phagocytosis by blocking the lysosome from moving to the phagosome.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a bacterium resisting phagocytosis by blocking the lysosome from moving to the phagosome.
Certain bacteria block the vesicular transport machinery that enables the phagosome to fuse with the lysosome. Since the lysosome does not fuse with the phagosome, the ingested bacteria are not killed.

- f. Some bacteria, such as pathogenic *Mycobacterium* and *Legionella pneumophila*, **prevent the acidification of the phagosome which is needed for effective killing of microbes by lysosomal enzymes.** (Normally after the phagosome forms, the contents become acidified because the lysosomal enzymes used for killing function much more effectively at an acidic pH.)

Flash animation showing a bacterium preventing acidification of the phagosome following ingestion.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a bacterium preventing acidification of the phagosome following ingestion.
During phagosome formation, an electron pump brings protons (H ⁺) into the phagosome. This lowers the pH within the phagosome so that when a lysosome fuses with the phagosome, the pH is correct for the acid hydrolases to effectively break down cellular proteins. Some bacteria prevent the acidification of the phagosome that is needed for effective killing of microbes by lysosomal enzymes.

- g. The carotenoid pigments that give *Staphylococcus aureus* its golden color and group B streptococci (GBS) its orange tint **shield the bacteria from the toxic oxidants that neutrophils use to kill bacteria.**
- h. Cell wall lipids of *Mycobacterium tuberculosis*, such as lipoarabinomannan, **arrest the maturation of phagosomes preventing delivery of the bacteria to lysosomes.**
- i. Some bacteria are able to **kill phagocytes.** Bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* produce the exotoxin **leukocidin** which damages the cytoplasmic membrane of the phagocyte. On the other hand, bacteria, such as *Shigella* and *Salmonella*, **induce macrophage apoptosis**, a programmed cell death.

Flash animation showing a bacterium using leukocidin to kill a phagocyte.
Copyright © Gary E. Kaiser

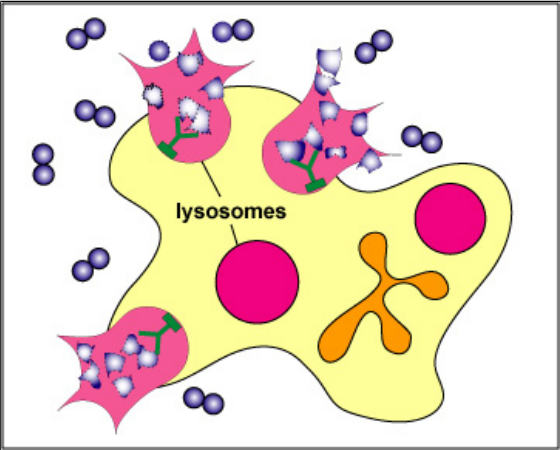
html5 version of animation for iPad showing a bacterium using leukocidin to kill a phagocyte.

Bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* produce the type III toxin leukocidin that damages the cytoplasmic membrane of the phagocyte resulting in its death.

If the the infection site contains very large numbers of microorganisms and high levels of inflammatory cytokines and chemokines are being produced in response to PAMPs , 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 (see Fig. 15).**

Fig. 15: Extracellular Destruction of Bacteria by a Phagocyte

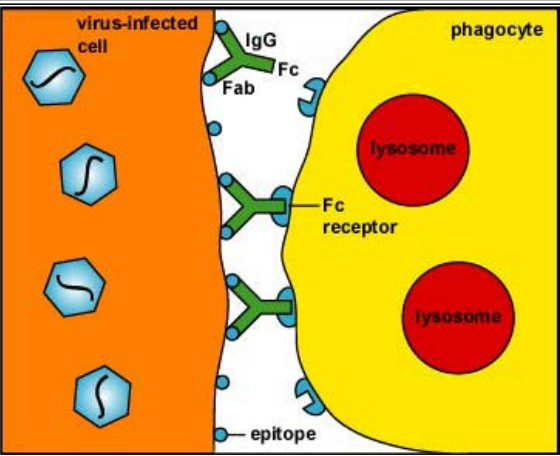
A diagram of a phagocyte, represented as a yellow irregular shape, with several pink, star-shaped structures labeled 'lysosomes' inside. Some of these lysosomes are shown with their contents being released into the extracellular space, where they are attacking small blue spherical bacteria. The phagocyte itself has a large pink nucleus and an orange, branching structure representing the Golgi apparatus.

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The contents of the lysosomes are dumped by the phagocyte in order to kill the microbes extracellularly. As a result, however, surrounding host cells and tissues are also destroyed.

The phagocyte will also empty the contents of its lysosomes for extracellular killing if the cell to which the phagocyte adheres is too large to be engulfed (**see Fig. 16 and Fig. 17**).

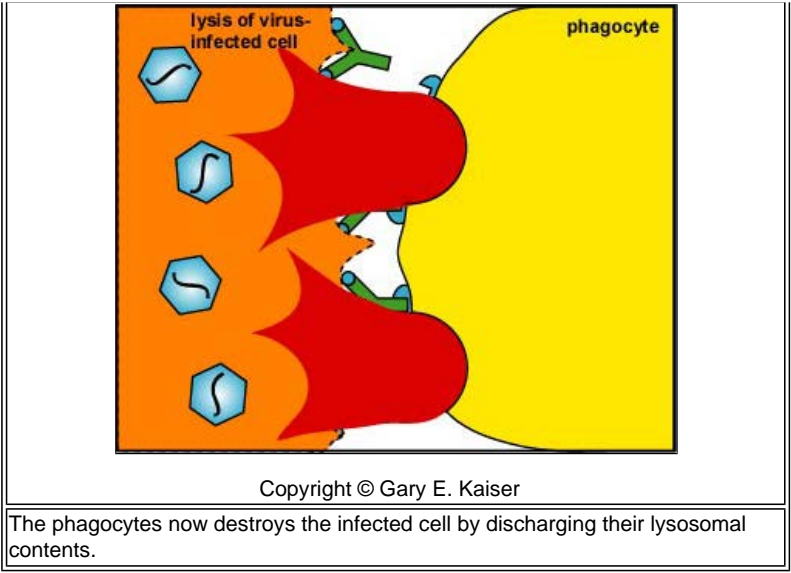
Fig. 16: Attachment of Phagocytes to Virus-Infected Cells by Opsonization, Step-1

A diagram illustrating the first step of opsonization. On the left is an orange 'virus-infected cell' with several blue, hexagonal 'epitope' structures on its surface. On the right is a yellow 'phagocyte' containing two red 'lysosome' structures. Green Y-shaped 'IgG' molecules are shown with their 'Fab' arms bound to the epitopes on the infected cell and their 'Fc' arms bound to 'Fc receptor' proteins on the phagocyte's surface.

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The Fab portions of IgG or C3b and C4b from the complement pathways bind to viral epitopes on virus-infected cells. This enables phagocytes to attach to the infected cell by way of their Fc and C3b/C4b receptors.

Fig. 17: Attachment of Phagocytes to Virus-Infected Cells by Opsonization, Step-2



Flash animation summarizing extracellular killing by phagocytosis.
Copyright © Gary E. Kaiser
html5 version of animation for iPad summarizing extracellular killing by phagocytosis.

There are **2 killing systems** in neutrophils and macrophages: the oxygen-dependent system and the oxygen-independent system.

1. The oxygen-dependent system: production of reactive oxygen species (ROS)

The cytoplasmic membrane of phagocytes contains the enzyme oxidase which converts oxygen into **superoxide anion (O_2^-)**. This can combine with water by way of the enzyme dismutase to form **hydrogen peroxide (H_2O_2)** and **hydroxyl (OH) radicals**.

In the case of neutrophils, but not macrophages, the hydrogen peroxide can then combine with chloride (Cl^{2-}) ions by the action of the enzyme myeloperoxidase (MPO) to form **hypochlorous acid (HOCL)**, and **singlet oxygen**.

In macrophages, nitric oxide (NO) can combine with hydrogen peroxide to form peroxynitrite radicals. (In addition to ROS and NO, macrophages secrete inflammatory cytokines such as TNF-alpha, IL-1, IL-8, and IL-12 to promote an inflammatory response.)

These compounds are very microbicidal because they are powerful **oxidizing agents** which oxidize most of the chemical groups found in proteins, enzymes, carbohydrates, DNA, and lipids. Lipid oxidation can break down cytoplasmic membranes. Collectively, these oxidizing free radicals are called **reactive oxygen species (ROS)**.

Oxidase also acts as an electron pump that brings protons (H^+) into the phagosome. This **lowers the pH within the phagosome** so that when lysosomes fuse with the phagosome, the pH is correct for the acid hydrolases, like elastase, to effectively **break down cellular proteins**.

In addition to phagocytes using this oxygen-dependant system to kill microbes intracellularly, **neutrophils also routinely release these oxidizing agents, as well as acid hydrolases, for the purpose of killing microbes extracellularly**. These agents, however, also wind up killing the neutrophils themselves as well as some surrounding body cells and tissues as mentioned above.

2. The oxygen-independent system

Some lysosomes contain defensins, cationic peptides that alter cytoplasmic membranes; lysozyme, an enzyme that breaks down peptidoglycan, lactoferrin, a protein that deprives bacteria of needed iron; cathepsin G, a protease that causes damage to microbial membranes; elastase, a protease that kills many types of bacteria; cathelicidins, proteins that upon cleavage are directly toxic to a variety of microorganisms; bactericidal permeability inducing protein (BPI), proteins used by neutrophils to kill certain bacteria by damaging their membranes; collagenase; and various other digestive enzymes that exhibit antimicrobial activity by breaking down proteins, RNA, phosphate compounds, lipids, and carbohydrates.

Concept Map for Phagocytosis

TPS Questions

Self Quiz for The Steps Involved in Phagocytosis

[Quiz Group](#)

[Return to Unit 5 and 6 Table of Contents](#)

[Back to Softchalk Lessons Table of Contents](#)

Early Induced Innate Immunity: Natural Killer Cells (NK Cells) and Invariant Natural Killer T-Lymphocytes (iNKT Cells)



1. Natural Killer (NK) cells are able to recognize infected cells, cancer cells, and stressed cells and kill them. In addition, they produce a variety of cytokines, including proinflammatory cytokines, chemokines, colony-stimulating factors, and other cytokines that function as regulators of body defenses.
2. When body cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell.
3. NK cells use a dual receptor system in determining whether to kill or not kill human cells.
4. The first receptor, called the killer-activating receptor, can bind to these stress-induced molecules, and this sends a positive signal that enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal.
5. The second receptor, called the killer-inhibitory receptor, recognizes MHC-I molecules that are usually present on all nucleated human cells. If MHC-I molecules/self peptide complexes are expressed on the cell, the killer-inhibitory receptors on the NK cell recognize this MHC-I/peptide complex and sends a negative signal that overrides the original kill signal and prevents the NK cell from killing the cell to which it has bound.
6. Viruses, stress, and malignant transformation can often interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell kills the cell to which it has bound.
7. NK cells kill their target cells by inducing apoptosis, a programmed cell suicide.
8. NK cells also play a role in adaptive immune responses by way of antibody-dependent cellular cytotoxicity or ADCC where they bind to and kill cells to which antibody molecules have bound.

9. *Invariant natural killer T-lymphocytes (iNKT cells) are a subset of lymphocytes that have T-cell receptors on their surface for glycolipid antigen recognition. They also have natural killer (NK) cell receptors.*
10. *Through the cytokines they produce, iNKT cells are able to promote and suppress different innate and adaptive immune responses. They also play a regulatory role in the development of autoimmune diseases, asthma, and transplantation tolerance. iNKT cell deficiency or dysfunction can lead to the development of autoimmune diseases, human asthma, and cancers.*

Common Course Objective

1. Describe the role of NK cells in innate immunity.

Detailed Learning Objectives

- 1**. Describe how NK cells are able to recognize and kill infected cells and cancer cells lacking MHC-I molecules.
2. State two factors that can result in a nucleated human cell not producing MHC-I molecules.
3. State how iNKT cells recognize glycolipids in order to become activated.
4. Describe the overall function of iNKT cells in terms how they promote both innate and adaptive immunity and may also help to regulate the immune responses.
- (**) = More depth and common theme

Early Induced Innate Immunity: Natural Killer Cells (NK Cells) and Invariant Natural Killer T-Lymphocytes (iNKT Cells)

A. Natural Killer (NK) Cells

NK cells are important in innate immunity because they are able to recognize infected cells, cancer cells, and stressed cells and kill them. In addition, they produce a variety of cytokines, including proinflammatory cytokines, chemokines, colony-stimulating factors, and other cytokines that function as regulators of body defenses. For example, through cytokine production NK cells also suppress and/or activate macrophages , suppress and/or activate the antigen-presenting capabilities of dendritic cells, and suppress and/or activate T-lymphocyte responses.

NK cells **use a dual receptor system in determining whether to kill or not kill human cells. When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules** such as MHC class I polypeptide-related sequence A (MICA) and MHC class I polypeptide-related sequence B (MICB) **are produced and are put on the surface of that cell.**

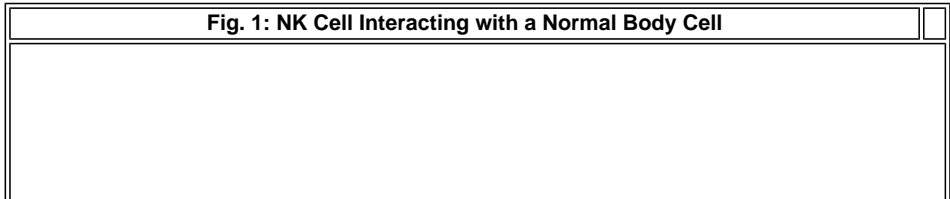
The first receptor, called the **killer-activating receptor**, **can bind to these stress-induced molecules, and this sends a positive signal that enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal.**

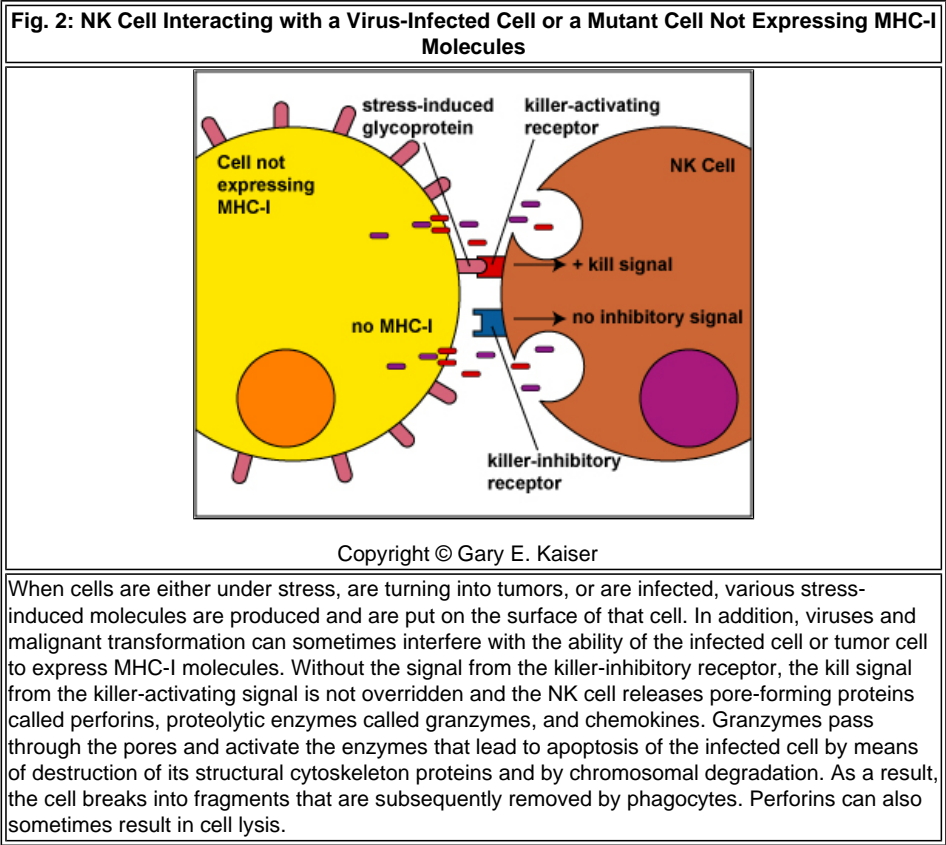
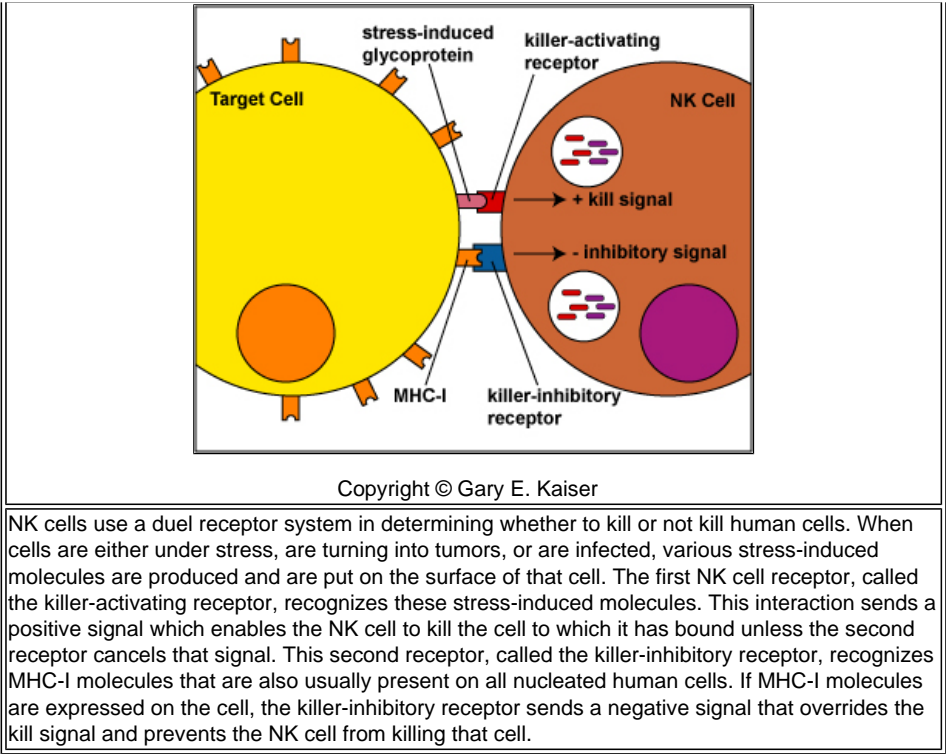
This second receptor, called the **killer-inhibitory receptor**, **recognizes MHC-I molecules that are usually present on all nucleated human cells.** MHC-I molecules, produced by all nucleated cells in the body, possess a deep groove that can bind peptides from proteins found within the cytosol of human cells, transport them to the surface of that cell, and display the MHC-I/peptide complex to receptors on cytotoxic T-lymphocytes or CTLs. If the MHC-I molecules have peptides from the body's own proteins bound to them, CTLs do not recognize those cells as foreign and the cell is not killed. If, on the other hand, the MHC-I molecules have peptides from viral, bacterial, or mutant proteins bound to them, CTLs recognize that cell as foreign and kill that cell. (CTLs will be discussed in greater detail in Unit 5.)

For More Information: Preview of cytotoxic T-lymphocytes (CTLs)

If MHC-I molecules/self peptide complexes are expressed on the cell, the killer-inhibitory receptors on the NK cell recognize this MHC-I/peptide complex and sends a negative signal that overrides the original kill signal and prevents the NK cell from killing the cell to which it has bound (see Fig. 1).

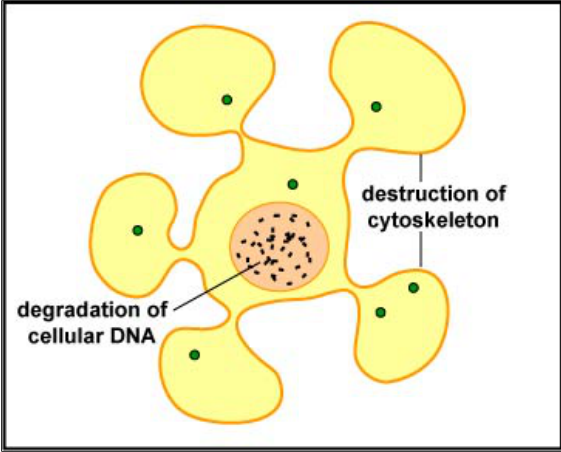
Viruses, stress, and malignant transformation, however, can often interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell kills the cell to which it has bound (see Fig. 2).





The NK cell then releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes (see Fig. 3). Perforins can also sometimes result in cell lysis.

Fig. 3: Apoptosis by NK Cells



The diagram shows a yellow NK cell with several green granules. It is interacting with a target cell (orange) that has a nucleus and a cytoskeleton. Labels indicate 'degradation of cellular DNA' and 'destruction of cytoskeleton'.

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Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell kills the cell to which it has bound. The NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

Flash animation of a NK cell interacting with a normal body cell.

Copyright © Gary E. Kaiser

html5 version of animation for iPad of a NK cell interacting with a normal body cell.

NK cells use a dual receptor system in determining whether to kill or not kill human cells. When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules are produced and are put on the surface of that cell. The first NK cell receptor, called the killer-activating receptor, recognizes these stress-induced molecules. This interaction sends a positive signal which enables the NK cell to kill the cell to which it has bound unless the second receptor cancels that signal. This second receptor, called the killer-inhibitory receptor, recognizes MHC-I molecules that are also usually present on all nucleated human cells. If MHC-I molecules are expressed on the cell, the killer-inhibitory receptor sends a negative signal that overrides the kill signal and prevents the NK cell from killing that cell.

Flash animation of a NK cell interacting with a virus-infected cell or tumor cell not expressing MHC-I molecules.

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html5 version of animation for iPad of a NK cell interacting with a virus-infected cell or tumor cell not expressing MHC-I molecules.

Viruses and malignant transformation can sometimes interfere with the ability of the infected cell or tumor cell to express MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is not overridden and the NK cell releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the infected cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation. As a result, the cell breaks into fragments that are subsequently removed by phagocytes. Perforins can also sometimes result in cell lysis.

Flash animation of apoptosis by NK cells

Copyright © Gary E. Kaiser

html5 version of animation for iPad of apoptosis by NK cells

NK cells release pore-forming proteins called perforins and proteolytic enzymes called granzymes. Granzymes pass through the pores and activate the enzymes that lead to apoptosis, a programmed suicide of the infected cell. Apoptosis occurs when certain granzymes activate a group of protease enzymes called caspases that destroy the protein structural scaffolding of the cell, degrade the cell's nucleoprotein, and activate enzymes that degrade the cell's DNA. As a result, the infected cell breaks into membrane-bound fragments that are subsequently removed by phagocytes. If very large numbers of perforins are inserted into the plasma membrane of the infected cell, this can result in a weakening of the membrane and lead to cell lysis rather than apoptosis. An

advantage to killing infected cells by apoptosis is that the cell's contents, including viable virus particles and mediators of inflammation, are not released as they are during cell lysis.

Cytokines such as interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) produced by T_H1 lymphocytes activate NK cells.

NK cells also play a role in adaptive immune responses. As will be seen in Unit 6, NK cells are also capable of **antibody-dependent cellular cytotoxicity or ADCC where they kill cells to which antibody molecules have bound.**

For more information: Preview of ADCC

Self Check

B. Invariant Natural Killer T-Lymphocytes (iNKT Cells)

iNKT cells are a subset of lymphocytes that bridge the gap between innate and adaptive immunity. They have T-cell receptors (TCRs) on their surface **for glycolipid antigen recognition**. They also have natural killer (NK) cell receptors.

Through the cytokines they produce once activated, iNKT cells are **essential in both innate and adaptive immune protection against pathogens and tumors. They also play a regulatory role in the development of autoimmune diseases, asthma, and transplantation tolerance.** It has been shown that **iNKT cell deficiency or disfunction can lead to the development of autoimmune diseases, human asthma, and cancers.**

Pathogens may not directly activate iNKT cells. **The TCR of iNKT cells recognize exogenous glycolipid antigens, as well as endogenous self glycolipid antigens presented by MHC-I-like CD1d molecules on antigen presenting dendritic cells.** iNKT cells can **also be activated by the cytokine interleukin-12 (IL-12) produced by dendritic cells that have themselves become activated by pathogen-associated molecular patterns (PAMPs) of microbes binding to the pattern-recognition receptors (PRRs) of the dendritic cell.**

Once activated, the iNKT cells rapidly produce **large quantities of cytokines**, including interferon-gamma (IFN- γ), interleukin-4 (IL-4), interleukin-2 (IL-2), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF- α), interleukin-13 (IL-13), and chemokines. **Through the rapid productions of such cytokines, iNKT cells are able to promote and suppress different innate and adaptive immune responses.** For example, large amounts of IFN- γ are produced by activated iNKT cells. IFN- γ activates NK cells and macrophages as a part of innate immunity.

It has been proposed that if the iNKT cell is repeatedly stimulated by the body's own glycolipids in the absence of microbes that this **might stimulate the iNKTcell /dendritic cell interaction to produce tolerizing signals that inhibit the T_H1 cell response and possibly stimulate the production of regulatory T-lymphocytes (T_{reg} cells).** In this way it might suppress autoimmune responses and prevent tissue damage.

There is also growing evidence that **early childhood exposure to microbes is associated with protection against allergic diseases, asthma, and inflammatory diseases such as ulcerative colitis.** It has been found that germ-free mice have large accumulations of mucosal iNKT cells in the lungs and intestines and increased morbidity from allergic asthma and inflammatory bowel disease. However, colonization of neonatal germ-free mice with normal microbiota resulted in mucosal iNKT cell tolerance to these diseases. It has been proposed that microbes the human body has been traditionally exposed to from early childhood throughout most of human history might play a role in developing normal iNKT cell numbers and iNKT cell responses.

iNKT cells will be discussed in further detail in Unit 6.

Concept Map for NK Cells and iNKT Cells

Self Quiz for Early Induced Innate Immunity: Natural Killer Cells (NK Cells) and Invariant Natural Killer T-Lymphocytes (iNKT Cells)

Quiz Group

[Return to Unit 5 and 6 Table of Contents](#)

[Back to Softchalk Lessons Table of Contents](#)

Early Induced Innate Immunity: Inflammation



1. Most of the body defense elements are located in the blood and inflammation is the means by which body defense cells and defense chemicals leave the blood and enter the tissue around the injured or infected site.
2. As part of the mechanism for inflammation, smooth muscles around larger blood vessels contract to slow the flow of blood through the capillary beds at the infected or injured site. This gives more opportunity for leukocytes to adhere to the walls of the capillary and squeeze out into the surrounding tissue.
3. As part of the mechanism for inflammation, the endothelial cells that make up the wall of the smaller blood vessels contract. This increases the space between the endothelial cells resulting in increased capillary permeability.
4. As part of the mechanism for inflammation, adhesion molecules are activated on the surface of the endothelial cells on the inner wall of the capillaries and corresponding molecules on the surface of leukocytes called integrins attach to these adhesion molecules allowing the leukocytes to flatten and squeeze through the space between the endothelial cells. This process is called diapedesis or extravasation.
5. As part of the mechanism for inflammation, activation of the coagulation pathway causes fibrin clots to physically trap the infectious microbes and prevent their entry into the bloodstream.
6. Acute inflammation is essential to body defense.
7. As a result of this increased permeability, plasma flows out of the blood into the tissue delivering clotting factors, antibody molecules, complement pathway proteins, nutrients, antibacterial enzymes and peptides, and transferrin for innate body defense.
8. As a result of this increased permeability, leukocytes enter the tissue delivering phagocytic cells, inflammation-inducing cells, cytotoxic T-lymphocytes, effector T4-lymphocytes, and NK cells.
9. Inflammatory cytokines also, enable endothelial cells form a fine network of new capillaries into the injured area to supply blood, oxygen, and nutrients to the inflamed tissue, and enable fibroblasts to deposit the protein collagen in the injured area and form a bridge of connective scar tissue

- to close the open, exposed area.
10. Chronic inflammation can result in considerable tissue damage and scarring, primarily to extracellular killing by phagocytes and hypoperfusion.
11. Chronic inflammation is thought to also contribute to heart disease, Alzheimer's disease, diabetes, and cancer.

Common Course Objective

- 1. Explain the physiological significance of localized inflammation and systemic inflammation.
- 2. Describe the mechanism of inflammation, indicating the various beneficial effects associated with plasma leakage and diapedesis.

Detailed Learning Objectives

- 1**. Describe the 4 processes that make up the inflammatory **mechanism**.
 - 2**. Briefly describe the various **beneficial effects of inflammation** that are associated with plasma leakage and with diapedesis.
 - 3**. Briefly describe the process of diapedesis, indicating the role of P-selectins, integrins, and adhesion molecules.
 - 4. Briefly describe the healing stage of inflammation.
 - 5. Briefly describe the problems that arise from chronic inflammation.
- (**) = More depth and common theme

TPS Questions

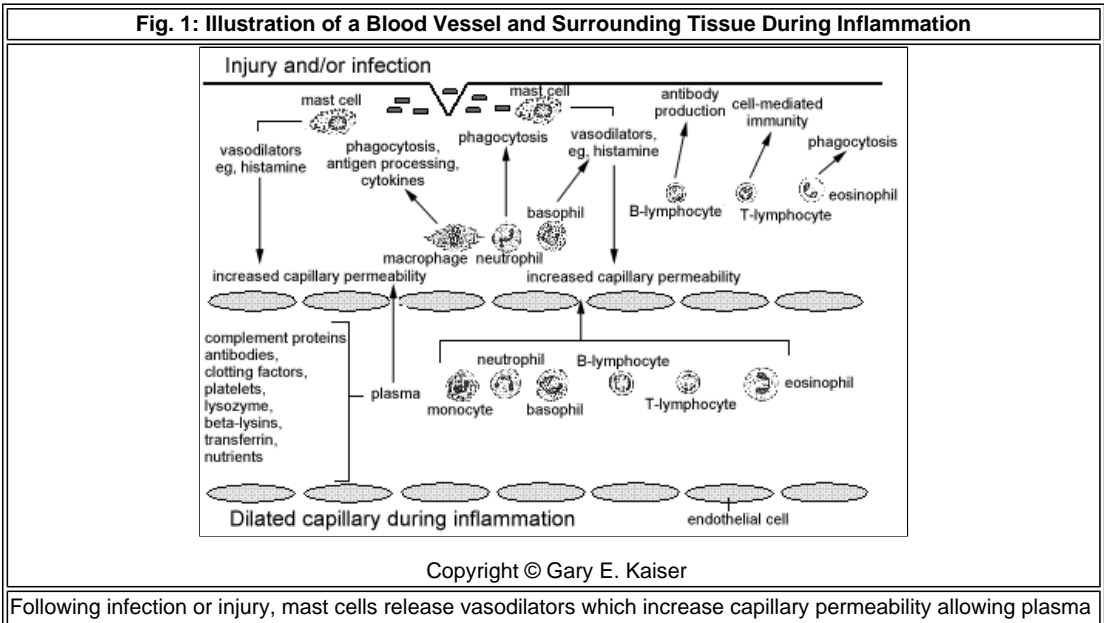
Early Induced Innate Immunity: Inflammation

The inflammatory response is an attempt by the body to restore and maintain homeostasis after injury and is an integral part of body defense. **Most of the body defense elements are located in the blood and inflammation is the means by which body defense cells and defense chemicals leave the blood and enter the tissue around the injured or infected site.** Inflammation is essentially beneficial, however, excess or prolonged inflammation can cause harm.

1. The Mechanism of Inflammation

Essentially, four processes make up the inflammatory mechanism:

- a. **Smooth muscles around larger blood vessels contract to slow the flow of blood through the capillary beds at the infected or injured site.** This gives **more opportunity for leukocytes to adhere to the walls of the capillary** and squeeze out into the surrounding tissue.
- b. **The endothelial cells that make up the wall of the smaller blood vessels contract. This increases the space between the endothelial cells resulting in increased capillary permeability.** Since these blood vessels get larger in diameter as a result of this, the process is called **vasodilation**. (see Fig. 1).



- and leukocytes to leave the bloodstream and enter the tissue. Benefits are as follows:
1. Plasma entering the tissue delivering:
 - a. Complement proteins - results in inflammation, enhanced attachment, WBC chemotaxis, cell lysis.
 - b. Antibodies - results in opsonization, cell lysis, neutralization of viruses and toxins, agglutination of microbes, blocking microbial adherence, immobilization of microbes.
 - c. Clotting factors and platelets - results localization of infection, WBC chemotaxis, stoppage of bleeding.
 - d. Lysozyme - breaks down peptidoglycan.
 - e. Human defensins - Damages bacterial cytoplasmic membranes.
 - f. Transferrin - Deprives bacteria of iron.
 2. Leukocytes enter tissue
 - a. Neutrophils - results in phagocytosis, enzymes to synthesize inflammation mediators.
 - b. Eosinophils - results in phagocytosis, moderation of inflammatory damage.
 - c. Basophils - results in release histamine, promote inflammation.
 - d. Monocytes - become macrophages - results in phagocytosis, process antigens, release cytokines.
 - e. B-lymphocytes - results in antibody production.
 - f. T-lymphocytes - release cytokines, become cytotoxic T-lymphocytes CTLs).

Scanning electron micrographs of a cross section of a capillary showing an endothelial cell and a capillary with a red blood cell; courtesy of Dennis Kunkel's Microscopy).

Illustration of a arterioles, venules, and a capillary bed.

Animation showing a capillary prior to vasodilation.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a capillary prior to vasodilation.
White blood cells and plasma flowing through a venule prior to vasodilation.

Animation showing vasodilation.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing vasodilation.
Most leukocyte diapedesis (extravasation) occurs in post-capillary venules because hemodynamic shear forces are lower in these venules. This makes it easier for leukocytes to attach to the inner wall of the vessel and squeeze out between the endothelial cells.
Following infection or injury, vasodilators are released that increase venule permeability. Constriction of the endothelial cells of the venules allows for diapedesis (extravasation), during which defense white blood cells such as neutrophils and monocytes leave the blood and enter the tissue around capillary beds where they are chemotactically attracted to the infection site. In addition, plasma leaves the bloodstream and enters the tissue delivering defense chemicals such as antibodies, complement proteins, and clotting factors.

- c. Molecules called **selectins** are produced on the membrane of the leukocyte and are able to reversibly **bind to corresponding selectin glycoprotein receptors** on the inner wall of the venule. This reversible binding enables the leukocyte to **roll along the inner wall of the venule**. **Adhesion molecules are activated on the surface of the endothelial cells on the inner wall of the capillaries**. Corresponding **molecules on the surface of leukocytes called integrins attach to these adhesion molecules** allowing the leukocytes to flatten and **squeeze through the spaces between the endothelial cells**. This process is called **diapedesis or extravasation**.
- d. **Activation of the coagulation pathway causes fibrin clots to physically trap the infectious microbes and prevent their entry into the bloodstream**. This also triggers blood clotting within the surrounding small blood vessels to both stop bleeding and further prevent the microorganisms from entering the bloodstream.

3D animation illustrating illustrating white blood cells leaving capillaries and entering tissue (diapedesis) as well as the endomembrane system in the leukocyte.

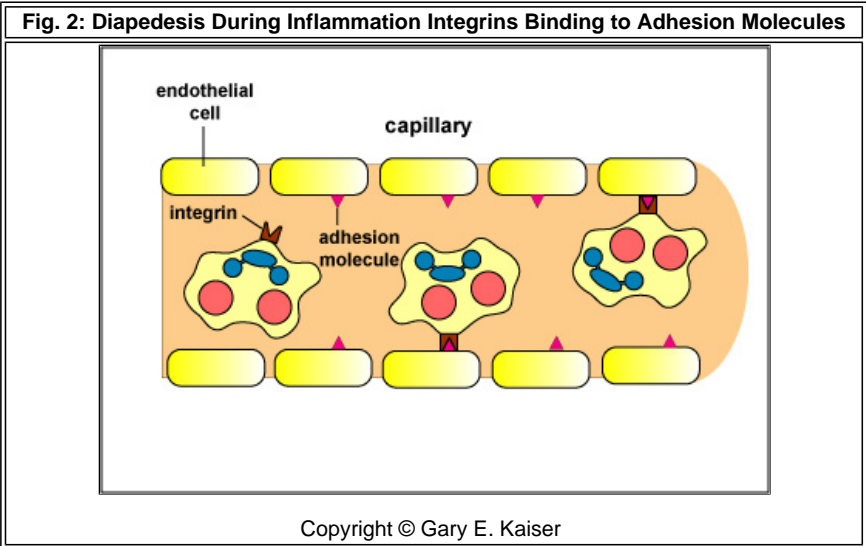
From Harvard University, The Inner Life of the Cell.

These four events are triggered and enhanced by a variety of chemical **inflammatory mediators**. We will now divide the inflammatory response into two stages: early inflammation and late inflammation.

a. Early Inflammation and Diapedesis

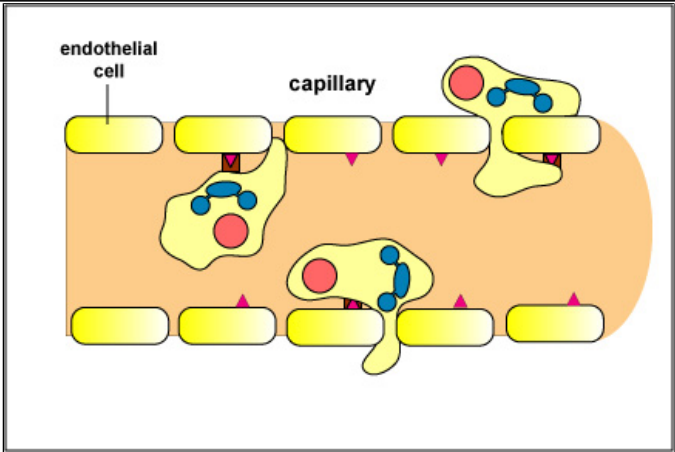
Most leukocyte diapedesis (extravasation) occurs in **post-capillary venules** because hemodynamic shear forces are lower in these venules. This makes it easier for leukocytes to attach to the inner wall of the vessel and squeeze out between the endothelial cells. We will look at this process in more detail below.

- 1) During the very early stages of inflammation, stimuli such as injury or infection trigger the release of a variety of mediators of inflammation such as leukotrienes, prostaglandins, and histamine. The binding of these mediators to their receptors on endothelial cells **leads to vasodilation, contraction of endothelial cells, and increased blood vessel permeability**. In addition, the basement membrane surrounding the capillaries becoming rearranged so as to promote the migration of leukocytes and the movement of plasma macromolecules from the capillaries into the surrounding tissue.
- Mast cells in the connective tissue as well as basophils, neutrophils and platelets leaving the blood from injured capillaries, release or stimulate the synthesis of vasodilators such as histamine, leukotrienes, kinins, and prostaglandins. Certain products of the complement pathways (C5a and C3a) can bind to mast cells and trigger their release their vasoactive agents. In addition, tissue damage activates the coagulation cascade and production of inflammatory mediators like bradykinins.
- 2) The binding of histamine to histamine receptors on endothelial cells triggers an upregulation of P-selectin molecules and platelet-activating factor or PAF on the endothelial cells that line the venules.
- 3). **The P-selectins then are able to reversibly bind to corresponding P-selectin glycoprotein ligands (PSGL-1) on leukocytes**. This reversible binding **enables the leukocyte to now roll along the inner wall of the venule**.
- 4) The binding of PAF to its corresponding receptor PAF-R on the leukocyte upregulates the surface expression of an integrin called leukocyte function-associated molecule-1 (LFA-1) on the surface of the leukocyte.
- 5) The LFA-1 molecules on the rolling leukocytes **can now bind firmly to an an adhesion molecule** called intercellular adhesion molecule-1 (ICAM-1) **found on the surface of the endothelial cells** forming the inner wall of the blood vessel **(see Fig. 2)**.
- 6) **The leukocytes flatten out, squeeze between the constricted endothelial cells, and use enzymes to breakdown the matrix that forms the basement membrane surrounding the blood vessel**. The **leukocytes then migrate towards chemotactic agents** such as the complement protein C5a and leukotriene B₄ generated by cells at the site of infection or injury **(see Fig. 3)**.



Integrins on the surface of the leukocyte bind to adhesion molecules on the inner surface of the vascular endothelial cells.

Fig. 3: Diapedesis During Inflammation: Leukocytes Leaving the Blood vessel



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The leukocytes flatten out and squeeze between the endothelial cells to leave the blood vessels and enter the tissue. The increased capillary permeability also allows plasma to enter the tissue.

Animation summarizing early inflammation and diapedesis.

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html5 version of animation for iPad summarizing early inflammation and diapedesis.

Most leukocyte diapedesis (extravasation) occurs in post-capillary venules because hemodynamic shear forces are lower in these venules. This makes it easier for leukocytes to attach to the inner wall of the vessel and squeeze out between the endothelial cells.

- 1) During the very early stages of inflammation, stimuli such as injury or infection trigger the release of a variety of mediators of inflammation such as leukotrienes, prostaglandins, and histamine. The binding of these mediators to their receptors on endothelial cells leads to vasodilation, contraction of endothelial cells, and increased blood vessel permeability. In addition, the basement membrane surrounding the capillaries becoming rearranged so as to promote the migration of leukocytes and the movement of plasma macromolecules from the capillaries into the surrounding tissue.
- 2) The binding of histamine to histamine receptors on endothelial cells triggers an upregulation of P-selectin molecules and platelet-activating factor (PAF) on the endothelial cells that line the venules.
- 3). The P-selectins then are able to reversibly bind to corresponding P-selectin glycoprotein ligands (PSGL-1) on leukocytes. This reversible binding enables the leukocyte to now roll along the inner wall of the venule.
- 4) The binding of PAF to its corresponding receptor PAF-R on the leukocyte upregulates the surface expression of leukocyte function-associated molecule-1 (LFA-1) on the surface of the leukocyte.
- 5) The LFA-1 molecules on the rolling leukocytes can now bind firmly to intercellular adhesion molecule-1 (ICAM-1) found on the surface of the endothelial cells forming the inner wall of the blood vessel.
- 6) The leukocytes flatten out, squeeze between the constricted endothelial cells, and move across the basement membrane as they are attracted towards chemotactic agents such as the complement protein C5a and leukotriene B₄ generated by cells at the site of infection or injury.

b. Late Inflammation and Diapedesis

1. Usually within two to four hours of the early stages of inflammation, **activated macrophages and vascular endothelial cells release inflammatory cytokines** such as TNF and IL-1 when their toll-like receptors bind pathogen-associated molecular patterns - molecular components associated with microorganisms but not found as a part of eukaryotic cells. This enables **vascular endothelial cells of nearby venules to increase their expression of adhesion molecules** such as P-selectins, E-selectins, intercellular adhesion molecules (ICAMs), and chemokines.

For more information: Review of pathogen-associated molecular patterns (PAMPs)

For more information: Review of pattern-recognition receptors (PRRs)

For more information: Review of cytokines

- 2) The binding of TNF and IL-1 to receptors on endothelial cells triggers and maintains the inflammatory response by upregulation the production of the adhesion molecule E-selectin and maintaining P-selectin expression on the endothelial cells that line the venules.
- 3). The E-selectins on the inner surface of the endothelial cells can now bind firmly to its corresponding integrin E-selectin ligand-1 (ESL-1) on leukocytes (**see Fig. 2**).
- 4) The leukocytes flatten out, squeeze between the constricted endothelial cells, and move across the basement membrane as they are are attracted towards chemokines such as interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) generated by cells at the site of infection or injury (**see Fig. 3**). Leakage of fibrinogen and plasma fibronectin then forms a molecular scaffold that enhances the migration and retention of leukocytes at the infected site.

Animation summarizing late inflammation and diapedesis.
Copyright © Gary E. Kaiser
html5 version of animation for iPad summarizing late inflammation and diapedesis.
Most leukocyte diapedesis (extravasation) occurs in post-capillary venules because hemodynamic shear forces are lower in these venules. This makes it easier for leukocytes to attach to the inner wall of the vessel and squeeze out between the endothelial cells.
1) Usually within two to four hours of the early stages of inflammation, tissue macrophages activated by local injury or infection release proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1).
2) The binding of TNF and IL-1 to receptors on endothelial cells triggers and maintains the inflammatory response by upregulation the production of E-selectin molecules and maintaining P-selectin expression on the endothelial cells that line the venules.
3). The E-selectins on the inner surface of the endothelial cells can now bind firmly to corresponding E-selectin ligand-1 (ESL-1) on leukocytes.
4) The leukocytes flatten out, squeeze between the constricted endothelial cells, and move across the basement membrane as they are are attracted towards chemokines such as interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) generated by cells at the site of infection or injury.

2. Benefits of Inflammation

As a result of this increased capillary permeability:

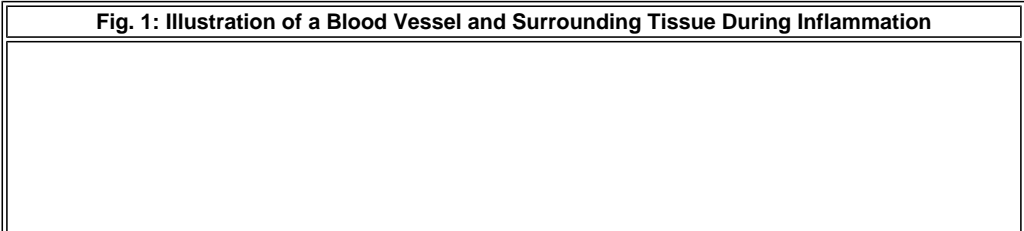
- a. **Plasma flows out of the blood into the tissue. Beneficial molecules in the plasma (see Fig. 1)** include:
1. **Clotting factors.** Tissue damage activates the coagulation cascade causing fibrin clots to form to localize the infection, stop the bleeding, and chemotactically attract phagocytes.
2. **Antibodies.** These help remove or block the action of microbes through a variety of methods that will be explained in Unit 6.

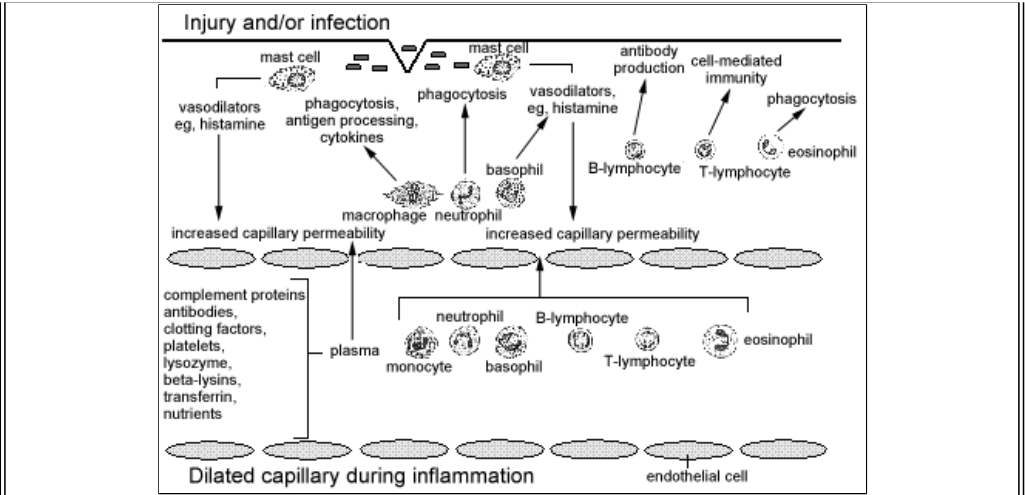
For more information: Preview of antibodies

3. **Proteins of the complement pathways.** These, in turn: 1) stimulate more inflammation (C5a, C3a, and C4a), 2) stick microorganisms to phagocytes (C3b and C4b), 3) chemotactically attract phagocytes (C5a), and 4) lyse membrane-bound cells displaying foreign antigens (membrane attack complex or MAC).

For more information: Review of the complement pathways

4. **Nutrients.** These feed the cells of the inflamed tissue.
5. **Lysozyme**, cathelicidins, phospholipase A₂, and **human defensins.** Lysozyme degrades peptidoglycan. Cathelicidins are cleaved into two peptides that are directly toxic to microbes and can neutralize LPS from the gram-negative bacterial cell wall. Phospholipase A₂ hydrolizes the phospholipids in the bacterial cytoplasmic membrane. Human defensins put pores in the cytoplasmic membranes of many bacteria. Defensins also activate cells involved in the inflammatory response.
6. **Transferrin.** Transferrin deprives microbes of needed iron.





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Following infection or injury, mast cells release vasodilators which increase capillary permeability allowing plasma and leukocytes to leave the bloodstream and enter the tissue. Benefits are as follows:

1. Plasma entering the tissue delivering:
 - a. Complement proteins - results in inflammation, enhanced attachment, WBC chemotaxis, cell lysis.
 - b. Antibodies - results in opsonization, cell lysis, neutralization of viruses and toxins, agglutination of microbes, blocking microbial adherence, immobilization of microbes.
 - c. Clotting factors and platelets - results localization of infection, WBC chemotaxis, stoppage of bleeding.
 - d. Lysozyme - breaks down peptidoglycan.
 - e. Human defensins - Damages bacterial cytoplasmic membranes.
 - f. Transferrin - Deprives bacteria of iron.
2. Leukocytes enter tissue
 - a. Neutrophils - results in phagocytosis, enzymes to synthesize inflammation mediators.
 - b. Eosinophils - results in phagocytosis, moderation of inflammatory damage.
 - c. Basophils - results in release histamine, promote inflammation.
 - d. Monocytes - become macrophages - results in phagocytosis, process antigens, release cytokines.
 - e. B-lymphocytes - results in antibody production.
 - f. T-lymphocytes - release cytokines, become cytotoxic T-lymphocytes CTLs)

b. **Leukocytes enter the tissue through a process called diapedesis or extravasation**, discussed above under early inflammation and late inflammation. **Benefits of diapedesis** include (see Fig. 1):

1. **Increased phagocytosis.** Neutrophils, monocytes that differentiate into macrophages when they enter the tissue, and eosinophils are phagocytic leukocytes that enter the tissue.
2. More **vasodilation.** Basophils, eosinophils, neutrophils, and platelets enter the tissue and release or stimulate the production of vasoactive agents that promote inflammation.
3. **Cytotoxic T-lymphocytes (CTLs), effector T4-lymphocytes, and NK cells** enter the tissue to kill cells such as infected cells and cancer cells that are displaying foreign antigens on their surface (discussed in Unit 5).

For more information: Review of leukocytes

Concept Map for Inflammation

Cytokines called **chemokines** are especially important in this part of the inflammatory response. They play key roles in diapedesis -enabling white blood cells to

adhere to the inner surface of blood vessels, migrate out of the blood vessels into the tissue, and be chemotactically attracted to the injured or infected site. They also **trigger extracellular killing by neutrophils**.

Finally, within 1 to 3 days, macrophages release the cytokines interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-a). These cytokines stimulate NK cells and T-lymphocytes to produce the cytokine interferon-gamma (IF-?). The (IF-?) then binds to receptors on macrophages causing them to produce **fibroblast growth factor and angiogenic factors for tissue remodeling**. With the proliferation of endothelial cells and fibroblasts, endothelial cells form a fine network of new capillaries into the injured area to supply blood, oxygen, and nutrients to the inflamed tissue. The fibroblasts deposit the protein collagen in the injured area and form a bridge of connective scar tissue to close the open, exposed area. This is called fibrosis or scarring, and represents the final healing stage.

Inflammation is normally carefully **regulated by cytokines**. Inflammatory cytokines such as interferon-gamma and interleukin-12 enhance the inflammatory response whereas the cytokine interleukin-10 inhibits inflammation by decreasing the expression of inflammatory cytokines.

So as can be seen, acute inflammation is essential to body defense. **Chronic inflammation**, however, can result in **considerable tissue damage and scarring**. With prolonged increased capillary permeability, neutrophils continually leave the blood and accumulate in the tissue at the infected or injured site. **As they discharge their lysosomal contents and reactive oxygen species or ROS, surrounding tissue is destroyed and eventually replaced with scar tissue**. Anti-inflammatory agents such as antihistamines or corticosteroids may have to be given to relieve symptoms or reduce tissue damage.

For example, as learned in Unit 3, during **severe systemic infections** with large numbers of microorganisms present, **high levels of** pathogen-associated molecular patterns (PAMPs) **are released** resulting in **excessive cytokine production** by macrophages and this can **harm the body**. In addition, neutrophils start releasing their proteases and **reactive oxygen species** that kill not only the bacteria, but the surrounding tissue as well. Harmful effects include high fever, hypotension, tissue destruction, wasting, acute respiratory distress syndrome or ARDS, disseminated intravascular coagulation or DIC, damage to the vascular endothelium, hypovolemia, and reduced perfusion of blood through tissues and organs resulting to shock, multiple system organ failure (MOSF), and often death. This excessive inflammatory response is referred to as **Systemic Inflammatory Response Syndrome or SIRS or the Shock Cascade**.

For more information: Review of the shock cascade during SIRS

TPS Questions

Chronic inflammation also contributes to heart disease, Alzheimer's disease, diabetes, and cancer.

- In the case of cancer, it is proposed that when macrophages produce inflammatory cytokines, such as TNF-alpha, these cytokines activate a gene switch in the cancer cell that turns on the synthesis of proteins that promote cell replication and inflammation while blocking apoptosis of the cancer cell.
- In heart disease, it is thought that macrophages digest low density lipoprotein or LDL, the bad cholesterol, and are then encased in a fibrous cap that forms arterial plaque.
- With diabetes, it is thought that the metabolic stress of obesity triggers innate immune cells and fat cells to produce cytokines such as TNF-alpha that can interfere with the normal function of insulin.
- In the case of Alzheimer's disease, microglial cells, macrophage-like cells in the brain, interact with the beta-amyloid proteins that build up in neurons of those with Alzheimer's and subsequently produce inflammatory cytokines and free radicals that destroy the neurons.

Concept Map for Inflammation

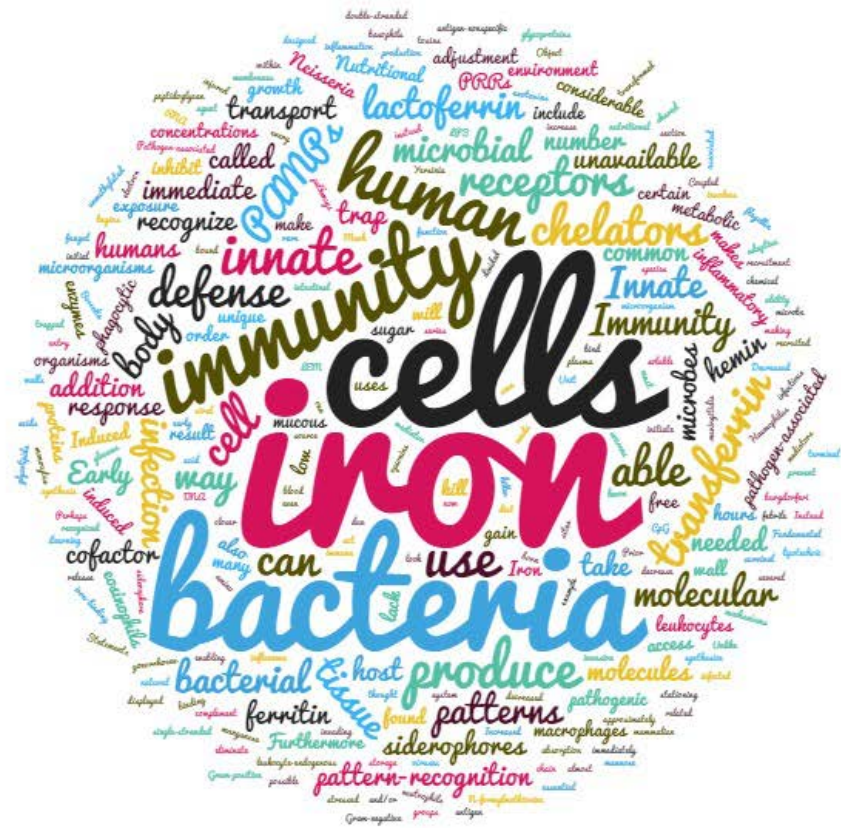
Self Quiz for Early Induced Innate Immunity: Inflammation



Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

Early Induced Innate Immunity: Nutritional Immunity



1. Iron is needed as a cofactor for certain enzymes in both bacteria and humans.
2. Both bacteria and human cells produce iron chelators that trap free iron from their environment and transport it into the cell.
3. During infection, the body makes considerable metabolic adjustment in order to make iron unavailable to microorganisms.
4. The lack of iron can inhibit the growth of many bacteria.
5. Some bacteria in addition to their own siderophores, produce receptors for iron chelators of other bacteria and/or human cells and in this way take iron being trapped for use by other organisms.
6. A number of bacteria are able to produce toxins that kill host cells only when iron concentrations are low and in this way gain access to the iron that was in those cells.

1. Describe at least 4 ways the body deprives microorganisms of iron.

Early Induced Innate Immunity: Nutritional Immunity

Iron is needed as a cofactor for certain enzymes in both bacteria and humans. Both bacteria and human cells produce iron chelators that trap free iron from their environment and transport it into the cell. **During infection, the body makes considerable metabolic adjustment in order to make iron unavailable to microorganisms.** Much of this is due to production of a defense chemical called **leukocyte-endogenous mediator (LEM)**. As a result of infection, there is:

1. **Decreased intestinal absorption of iron from the diet;**
2. **A decrease of iron in the plasma and an increase in iron in storage as ferritin;**
3. **An increased synthesis of the human iron-binding proteins (iron chelators) such as lactoferrin, transferrin, ferritin, and hemin that trap iron for use by human cells while making it unavailable to most microbes;**
4. Coupled with the febrile response, **decreased ability of bacteria to synthesize their own iron chelators** called siderophores;
5. **Prior stationing of lactoferrin at common sites of microbial invasion such as in the mucous of mucous membranes, and the entry of transferrin into the tissue during inflammation.**

This lack of iron, which is needed as a cofactor for certain enzyme reactions, can inhibit the growth of many bacteria.

As seen in Unit 3, some bacteria produce in addition to their own siderophore, receptors for siderophores of other bacteria in this way take iron from other bacteria. Furthermore, a number of pathogenic bacteria are able to bind human transferrin, lactoferrin, ferritin, and hemin and use that as their iron source. For example, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Haemophilus influenzae* are able to use iron bound to human transferrin and lactoferrin for their iron needs, while pathogenic *Yersinia* species are able to use transferrin and hemin as iron sources. *Borrelia burgdorferi* doesn't even use iron as a cofactor, but instead uses manganese. Furthermore, a number of bacteria are able to produce exotoxins that kill host cells only when iron concentrations are low. Perhaps in this way the bacteria can gain access to the iron that was in those cells.

Concept Map for Nutritional Immunity, the Acute Phase Response, Fever, Intraepithelial T-Cells, and B1-Cells
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[Quiz Group](#)

[Return to Unit 5 and 6 Table of Contents](#)

[Back to Softchalk Lessons Table of Contents](#)

Early Induced Innate Immunity: Fever



1. Activated macrophages and other leukocytes release inflammatory cytokines such as TNF-alpha, IL-1, and IL-6 when their pattern-recognition receptors (PRRs) bind pathogen associated molecular patterns or PAMPs.
2. These cytokines stimulate the anterior hypothalamus of the brain, the part of the brain that regulates body temperature, to produce prostaglandin E2, which leads to an increase bodily heat production and increased vasoconstriction.
3. Vasoconstriction decreases the loss of heat from the skin and increases body temperature.
4. Fever increases the environmental temperature above the optimum growth temperature for many microorganisms.
5. Fever leads to the production of heat shock proteins that are recognized by some intraepithelial T-lymphocytes resulting in the production of inflammation-promoting cytokines.
6. Fever elevates the temperature of the body increasing the rate of enzyme reactions, and speeding up metabolism within the body including that involved in innate and adaptive immunity as well as tissue repair.

1. Explain what cytokines are and their role in immunity.

Detailed Learning Objectives

- 1*. Describe the mechanism behind fever induction and indicate its possible benefits.
- 2. Define hyperpyrexia.

(*) = Common theme throughout the course

Early Induced Innate Immunity: Fever

Activated macrophages and other leukocytes release inflammatory cytokines such as TNF-alpha, IL-1, and IL-6 when their pattern-recognition receptors (PRRs) bind pathogen associated molecular patterns or PAMPs - molecular components associated with microorganisms but not found as a part of eukaryotic cells. These include bacterial molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, flagellin, and bacterial DNA. There are also pattern-recognition molecules for viral double-stranded RNA (dsRNA) and fungal cell walls components such as lipoteichoic acids, glycolipids, mannans, and zymosan.

For more information: Review of pathogen-associated molecular patterns (PAMPs)
For more information: Review of pattern-recognition receptors (PRRs)
For more information: Review of cytokines

These cytokines **stimulate the anterior hypothalamus of the brain**, the part of the brain that regulates body temperature, **to produce prostaglandin E2, which leads to an increase bodily heat production and increased vasoconstriction**. This, in turn, decreases the loss of heat from the skin and increases body temperature. Up to a certain point, fever is beneficial:

- 1. Fever **increases the environmental temperature above the optimum growth temperature for many microorganisms**. If the microorganisms are growing more slowly, the body's defenses have a better chance of removing them all.
- 2. Fever leads to the production of heat shock proteins that are recognized by some intraepithelial T-lymphocytes called delta gamma T-cells, resulting in the production of inflammation-promoting cytokines.
- 3. Fever **elevates the temperature of the body increasing the rate of enzyme reactions, and speeding up metabolism within the body**. An elevation in the rate of metabolism can increase the production and activity of phagocytes, speed up the multiplication of lymphocytes, increase the rate of antibody and cytokine production, increase the rate at which leukocytes are released from the bone marrow into the bloodstream, and speed up tissue repair.

Too high of a body temperature, however, may cause damage by denaturing the body's enzymes. **Hyperpyrexia is a fever with an extreme elevation of body temperature greater than or equal to 41.5 °C (106.7 °F)**. Body temperature this elevated often indicates a serious underlying condition and may lead to potentially hazardous side effects. As a result, hyperpyrexia is considered as a **medical emergency**.

Concept Map for Nutritional Immunity, the Acute Phase Response, Fever, Intraepithelial T-Cells, and B1-Cells
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Quiz Group

[Return to Unit 5 and 6 Table of Contents](#)

[Back to Softchalk Lessons Table of Contents](#)

Detailed Learning Objectives

- 1. Briefly describe the mechanism behind the acute phase response.
- 2*. State the functions of the following acute phase proteins:
 - a. C-reactive protein
 - b. mannose-binding lectin

(**) = More depth and common theme

Early Induced Innate Immunity: The Acute Phase Response

The acute phase response is an innate body defense seen during acute illnesses and involves the increased production of certain blood proteins termed **acute phase proteins**.

Activated macrophages and other leukocytes release inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), and interleukin-6 (IL-6) when their pattern-recognition receptors (PRRs) bind pathogen associated molecular patterns or PAMPs - molecular components associated with microorganisms but not found as a part of eukaryotic cells. These include bacterial molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, flagellin, pilin, and bacterial DNA. There are also pattern-recognition molecules for viral double-stranded RNA (dsRNA) and fungal cell walls components such as lipoteichoic acids, glycolipids, mannans, and zymosan.

For more information: Review of pathogen-associated molecular patterns (PAMPs)
For more information: Review of pattern-recognition receptors (PRRs)
For more information: Review of cytokines

These cytokines travel through the blood and **stimulate hepatocytes in the liver to synthesize and secrete acute phase proteins**. This response provides an early defense and enables the body to recognize foreign substances early on in the infection process prior to the full activation and implementation of the immune responses. Two important acute phase proteins are C-reactive protein and mannose-binding protein. **They function as soluble pattern-recognition receptors**.

- 1. **C-reactive protein (CRP)** binds to the phosphorylcholine portion of teichoic acids and lipopolysaccharides of bacterial and fungal cell walls. It also binds to the phosphocholine found on the surface of damaged or dead human cells. **It functions as an opsonin, sticking the microorganism to phagocytes, and activates the classical complement pathway** by binding C1q, the first component in the pathway.

For more information: Review of the complement pathways

- 2. **Mannan-binding lectin (MBL)** - also known as mannan-binding protein or MBP - binds to mannose-rich glycans (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar). These are common in microbial glycoproteins and glycolipids but rare in those of humans. **It functions as an opsonin, sticking the microorganism to phagocytes, and activates the lectin pathway**.

Flash animation showing activation of the lectin pathway, formation of C3 convertase, and formation of C5 convertase.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing activation of the lectin pathway, formation of C3 convertase, and formation of C5 convertase.
The lectin pathway is activated by the interaction of various microbial carbohydrates such as mannose, fucose, N-acetylglucosamine (NAG), lipoteichoic acids, and lipopolysaccharide (LPS) with mannose-binding lectin (MBL) or ficolins which forms complexes with two proteases called MASP1 and MASP2 in the plasma. (Lectins are carbohydrate-binding proteins.) The binding of the MBL (or the ficolin) to the microbial carbohydrate activates the associated MASP2 giving it the enzymatic activity to split C4 into C4a and C4b, as well as C2 into C2a and C2b.

Products of the complement pathways, in turn, **promote inflammation, attach microbes to phagocytes, cause to MAC cytolysis, and chemotactically attract phagocytes** to the infected area.

For more information: Review of the complement pathways

Concept Map for Nutritional Immunity, the Acute Phase Response, Fever, Intraepithelial T-Cells, and B1-Cells

Quiz Group

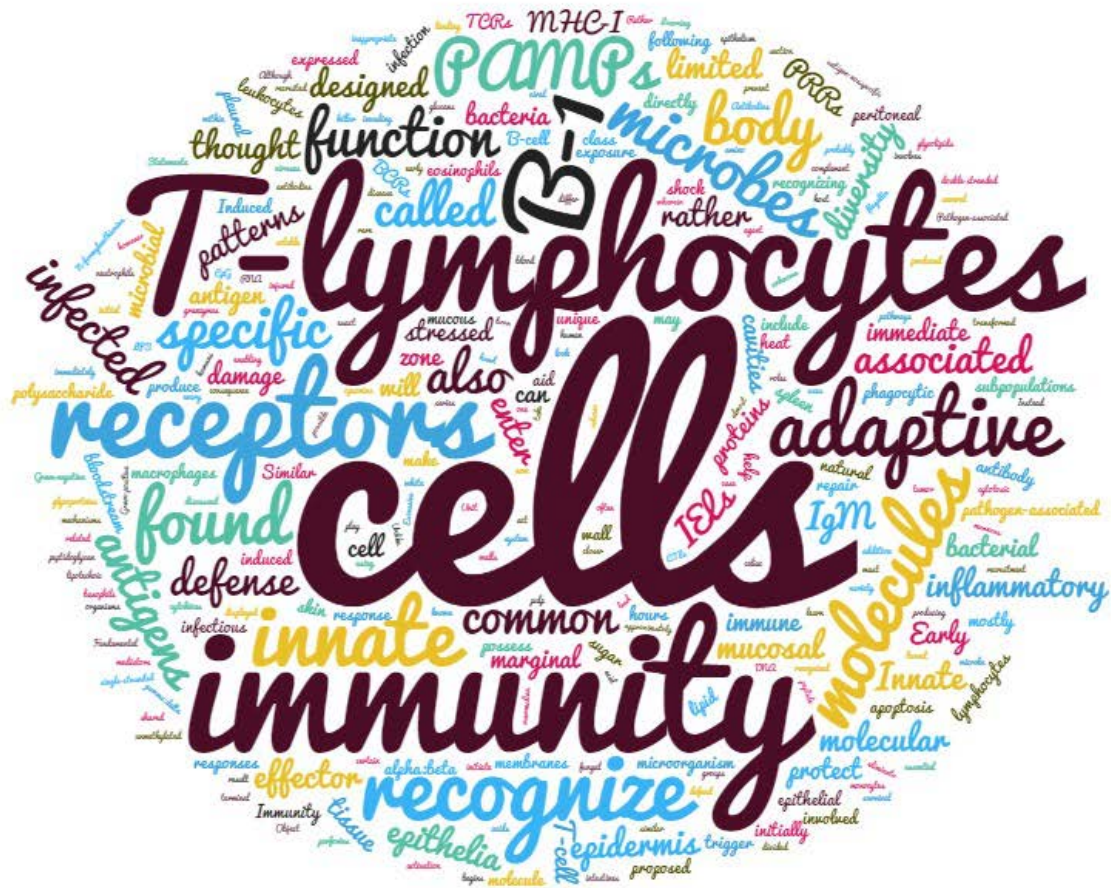


Return to Unit 5 and 6 Table of Contents

Back to Softchalk Lessons Table of Contents

Innate Immunity

Early Induced Innate Immunity: Intraepithelial T-Lymphocytes and B-1 Cells



Fundamental Statements for this Softchalk Lesson:

1. Most of the T-lymphocytes and B-lymphocytes in the body are involved in the adaptive immune responses wherein specific receptors on T-lymphocytes (T-cell receptors or TCRs) and B-lymphocytes (B-cell receptors or BCRs) recognize specific antigens of specific microbes.
2. Intraepithelial T-lymphocytes and B-1 cells, however, are subpopulations of T-lymphocytes and B-lymphocytes that possess a more limited diversity of receptors and are designed to directly recognize the more common microbes that enter the epidermis or the mucosal epithelia and function more as effector cells for innate immunity rather than adaptive immunity.
3. Intraepithelial T-lymphocytes (IELs) are found in the epidermis of the skin and the mucosal epithelia.
4. It has been proposed that they recognize molecules such as MHC-I molecules and heat shock proteins associated with epithelial cells but expressed only when those cells are infected and trigger apoptosis of these stressed or infected cells. They may also aid in repair of mucous membranes following inflammatory damage.
5. B-1 lymphocytes, or B-1 cells, are found mostly in the peritoneal and pleural cavities.
6. B-1 cells have a limited diversity of antigen receptors that initially produce a class of antibody molecule called IgM against common polysaccharide and lipid antigens of microbes and against PAMPs.
7. Similar B-lymphocytes called marginal zone B cells are found in the spleen and are thought to make IgM to protect against bacteria that enter the bloodstream.

Common Course Objective

- 1. Identify places where innate and adaptive immunity intersect.

Detailed Learning Objectives

- 1. Briefly describe how intraepithelial T-lymphocytes (gamma:delta T-lymphocytes) play a role in innate immunity.
- 2. Briefly describe how B-1 cells play a role in innate immunity.

Early Induced Innate Immunity: Intraepithelial T-Lymphocytes and B-1 Cells

Most of the T-lymphocytes and B-lymphocytes in the body are involved in the adaptive immune responses that will be discussed in Unit 6. In adaptive immunity, specific receptors on T-lymphocytes (T-cell receptors or TCRs) and B-lymphocytes (B-cell receptors or BCRs) recognize specific antigens of specific microbes.

For more information: Preview of B-lymphocytes
For more information: Preview of T4-lymphocytes
For more information: Preview of T8-lymphocytes

Intraepithelial T-lymphocytes and B-1 cells, on the other hand, are subpopulations of T-lymphocytes and B-lymphocytes that possess a more limited diversity of receptors and are designed to directly recognize the more common microbes that enter the epidermis or the mucosal epithelia. As such, they function more as effector cells for innate immunity rather than adaptive immunity.

- a. Intraepithelial T-lymphocytes (IELs) are found in the epidermis of the skin and the mucosal epithelia. These T-lymphocytes, known as gamma:delta T-lymphocytes, differ from the T-lymphocytes (alpha:beta T-lymphocytes) associated with adaptive immunity. The alpha:beta T-lymphocytes are designed to recognize peptide antigens bound to MHC-I molecules of infected cells and tumor cells.

Although their exact function is unknown, it has been proposed that they recognize molecules associated with epithelial cells but expressed only when those cells are infected, such as MHC-I molecules and heat shock proteins. They then trigger apoptosis of these stressed or infected cells using perforins and granzymes similar to cytotoxic T-lymphocytes (CTLs) of adaptive immunity. Rather than recognizing antigens specific to an infectious microorganism, they recognize molecules associated with the epithelium as a consequence of infection. Their T-cell receptors may also function as PRRs for recognizing certain PAMPs. As such, they function more as effector cells for innate immunity rather than adaptive immunity. They probably help defend the body by producing cytokines that play a variety of roles in body defense. IELs are also thought to aid in repair of mucous membranes following inflammatory damage. Excessive or inappropriate activation of IELs can also lead to damage of the intestines as in the case of celiac disease.
- b. B-1 lymphocytes, or B-1 cells are found mostly in the peritoneal and pleural cavities. B-1 cells have a limited diversity of antigen receptors that initially produce a class of antibody molecule called IgM against common polysaccharide and lipid antigens of microbes and against PAMPs. As such they function more as effector cells for innate immunity rather than adaptive immunity. Antibodies produced by B-1 cells are often called natural antibodies that help to protect against bacteria in body cavities. Similar B-lymphocytes called marginal zone B cells are found in the marginal zone of the white pulp of the spleen. These are thought to make IgM to protect against bacteria that enter the bloodstream.

Concept Map for Nutritional Immunity, the Acute Phase Response, Fever, Intraepithelial T-Cells, and B1-Cells
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[Return to Unit 5 and 6 Table of Contents](#)

[Back to Softchalk Lessons Table of Contents](#)

