11.3E: Phagocytosis

Skills to Develop

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

Phagocytic cells include neutrophils, eosinophils, monocytes, macrophages, dendritic cells, and B-lymphocytes. Phagocytosis is the primary method used by the body to remove free microorganisms in the blood and tissue fluids. The body's phagocytic cells are able to encounter these microorganisms in a variety of ways.
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 macrophages.

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 (including T4 and T8-Lymphocytes) to initiate adaptive immune responses.

Tissue fluid picks up microbes and then enters the lymph vessels as lymph. Lymph vessels carry the lymph to regional lymph nodes (Figure 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.

**Figure 1: Diagram of a Lymph Node.** Schematic diagram of a lymph node showing flow of lymph through lymph sinuses. Image used with permission (Public Domain; KC Panchal).

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.

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. 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).

The Steps Involved in Phagocytosis

There are a number of distinct steps involved in phagocytosis:

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

**Step 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.
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.

**Step 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 (Figure 2).

![Figure 2: Unenhanced Attachment of Bacteria to Phagocytes. 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.]

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 (Figure 3), 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.

**Figure 3:** Enhanced Attachment of Bacteria to Phagocytes. 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.
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 Figure 15 and Figure 16).

![Neutrophil NETs Trapping and Killing Bacteria](image)

Neutrophil NETs Trapping and Killing Bacteria. 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.

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 Figure 14). *Streptococcus pneumoniae* 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.

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.

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.

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 Figure 3). This is seen with the capsule of *Streptococcus pneumoniae*.

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 Figure 4) and in this way the bacteria become coated with antibodies in a way that does not result in opsonization (see Figure 5).

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**Step 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 Figure 6) and place it in an endocytic vesicle called a phagosome (see Figure 7).
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.

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 Figure 17). (This process is also used by eukaryotic cells to engulf and degrade unnecessary or dysfunctional cellular components such as damaged organelles.)

Some microorganisms are more resistant to phagocytic ingestion

a. Pathogenic *Yersinia*, such as the one that causes plague, contact phagocytes and, by means of a type III secretion system, deliver proteins which depolymerize the actin microfilaments needed for phagocytic engulfment into the phagocytes (see Figure 8). Another *Yersinia* protein degrades C3b and C5a.

b. Some bacteria, like *Mycobacterium tuberculosis*, *Salmonella*, and *Listeria monocytogenes* can block autophagy.
Blocking Phagosome Formation by Depolymerizing Actin. Molecules of some bacteria, through a type III secretion system, deliver proteins which depolymerize the phagocyte’s actin microfilaments used for phagocytic engulfment.

Step 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 Figure 9).

To view an electron micrograph of a phagolysosome, see the Web page for the University of Illinois College of Medicine.

Flash animation showing intracellular destruction.

html5 version of animation for iPad showing intracellular destruction.

3D animation illustrating organelles moving along a microtubule.

From Graham Johnson, Fifth Element. This animation takes some time to download.

Flash animation summarizing phagocytosis by unenhanced attachment.

html5 version of animation for iPad summarizing phagocytosis by unenhanced attachment.

Flash animation summarizing phagocytosis by enhanced attachment (opsonization).
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 (see Figure 10).

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

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.

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.

html5 version of animation for iPad showing a bacterium preventing acidification of the phagosome following ingestion.

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.

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

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 Figure 11).

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 Figure 12 and Figure 13).

Flash animation summarizing extracellular killing by phagocytosis.

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 (O²⁻). This can combine with water by way of the enzyme dismutase to form hydrogen peroxide (H₂O₂) and hydroxyl (OH) radicals.

In the case of neutrophils, but not macrophages, the hydrogen peroxide can then combine with chloride (Cl⁻) 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.

Exercise: Think-Pair-Share Questions

1. *Streptococcus pyogenes* has a capsule made of hyaluronic acid, a polysaccharide also found on human cells.
   
   Describe what significance this has in the bacterium resisting phagocytosis and why.

2. *Streptococcus pyogenes* produces a protein called protein G that binds to the Fc portion of human IgG.
   
   Describe what significance this has in the bacterium resisting phagocytosis and why.

3. Many bacteria produces capsules that cover their cell wall.
Describe what significance this has in the bacterium resisting phagocytosis and why.

4. People born with chronic granulomatious disease have neutrophils that lack the enzyme oxidase in their cytoplasmic membrane.

Describe what significance this has in the bacterium resisting phagocytosis and why.

Summary

Phagocytosis is the primary method used by the body to remove free microorganisms in the blood and tissue fluids. 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. Microorganisms entering lymph nodes 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. 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.

Dendritic cells located throughout the epithelium of the skin, the respiratory tract, and the gastrointestinal tract phagocytize 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. 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. 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).

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 lowers 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.

Contributors

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