2.3B: The Gram-Negative Cell Wall

Skills to Develop

2. Describe the composition of a Gram-negative cell wall and indicate the possible beneficial functions to the bacterium of peptidoglycan, the outer membrane, lipopolysaccharides, porins, and surface proteins.
3. Briefly describe how LPS and other PAMPs of the Gram-negative cell wall can promote inflammation.
4. State the function of bacterial adhesins, secretion systems, and invasins.
5. Define periplasm.
6. Define antigen and epitope.

Highlighted Bacterium

1. Read the description of *Escherichia coli*, and match the bacterium with the description of the organism and the infection it causes.

Highlighted Disease: Urinary Tract Infections (UTIs)

1. Define the following:
   a. urethritis
   b. cystitis
   c. pyelonephritis
2. Name at least 4 risk factors for UTIs.
3. Name the most common bacterium to cause UTIs; name at least 3 other bacteria that commonly cause UTIs.
4. Name at least 3 common symptoms of UTIs.
We will now look at the Gram-negative bacterial cell wall. As mentioned in the previous section on peptidoglycan, Gram-negative bacteria are those that decolorize during the Gram stain procedure, pick up the counterstain safranin, and appear pink (Figure 2B.1).

![Gram Stain of Escherichia coli. Note Gram-negative (pink) bacilli.](image)

Common Gram-negative bacteria of medical importance include *Salmonella* species, *Shigella* species, *Neisseria gonorrhoeae, Neisseria meningitidis, Haemophilus influenzae, Escherichia coli, Klebsiella pneumoniae, Proteus species, and Pseudomonas aeruginosa*.

Highlighted Bacterium: *Escherichia coli*

**Organism**

- *Escherichia coli* is a moderately-sized Gram-negative bacillus.
- Possess a peritrichous arrangement of flagella.
- Facultative anaerobe.

**Habitat**

- Normal flora of the intestinal tract in humans and animals.

**Source**

- Usually the patient's own fecal flora; some transmission is patient-to-patient.

**Clinical Disease**

- *E. coli* causes around 80 percent of all uncomplicated urinary tract infections (UTIs) and more than 50 percent of nosocomial UTIs. UTIs account for more than 7,000,000 physician office visits per year in the U.S. Between 35 and 40 percent of all nosocomial infections, about 900,000 per year in the U.S., are UTIs and are usually associated with urinary catheterization.
- *E. coli* causes wound infections, usually a result of fecal contamination of external wounds or a result of wounds...
that cause trauma to the intestinal tract, such as surgical wounds, gunshot wounds, knife wounds, etc.

- *E. coli* is by far the most common Gram-negative bacterium causing sepsis. **Septicemia** is a result of bacteria getting into the blood. They are usually introduced into the blood from some other infection site, such as an infected kidney, wound, or lung. There are approximately 500,000 cases of septicemia per year in the U.S. and the mortality rate is between 20 and 50 percent. Approximately 45 percent of the cases of septicemia are due to Gram-negative bacteria. *Klebsiella, Proteus, Enterobacter, Serratia*, and *E. coli*, are all common gram-negative bacteria causing septicemia.

- *E. coli*, along with group B streptococci, are the leading cause of **neonatal meningitis**.

- While *E. coli* is one of the dominant normal flora in the intestinal tract of humans and animals, some strains can cause **gastroenteritis**, an infection of the intestinal tract.
  
  - Enterotoxigenic *E. coli* (ETEC) produce enterotoxins that cause the loss of sodium ions and water from the small intestines resulting in a watery diarrhea. Over half of all travelers’ diarrhea is due to ETEC; almost 80,000 cases a year in the U.S.
  
  - Enteropathogenic *E. coli* (EPEC) cause an endemic diarrhea in areas of the developing world, especially in infants younger than 6 months. The bacterium disrupts the normal microvilli on the epithelial cells of the small intestines resulting in maladsorption and diarrhea.
  
  - Enteroaggregative *E. coli* (EAEC) is a cause of persistent diarrhea in developing countries. It probably causes diarrhea by adhering to mucosal epithelial cells of the small intestines and interfering with their function.
  
  - Enteroinvasive *E. coli* (EIEC) invade and kill epithelial cells of the large intestines causing a dysentery-type syndrome similar to *Shigella* common in underdeveloped countries.
  
  - Enterohemorrhagic *E. coli* (EHEC), such as *E. coli* 0157:H7, produce a shiga-like toxin that kills epithelial cells of the large intestines causing hemorrhagic colitis, a bloody diarrhea. In rare cases, the shiga-toxin enters the blood and is carried to the kidneys where, usually in children, it damages vascular cells and causes hemolytic uremic syndrome. *E. coli* 0157:H7 is thought to cause more than 20,000 infections and up to 250 deaths per year in the U.S.
  
  - Diffuse aggregative *E. coli* (DAEC) causes watery diarrhea in infants 1-5 years of age. They stimulate elongation of the microvilli on the epithelial cells lining the small intestines.


- Flash animation illustrating the interaction of the Gram’s stain reagents at a molecular level © Daniel Cavanaugh, Mark Keen, authors, Licensed for use, ASM MicrobeLibrary.

- Highlighted Infection: Urinary Tract Infections (UTIs)

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**Structure and Composition of the Gram-Negative Cell Wall**

In electron micrographs, the Gram-negative cell wall (Figures 1) appears multilayered. It consists of a thin, inner wall composed of peptidoglycan and an outer membrane.

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<thead>
<tr>
<th>outer membrane</th>
<th>peptidoglycan</th>
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<td>cytoplasm</td>
<td>cytoplasmic membrane</td>
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The Gram-negative cell wall is composed of a thin, inner layer of peptidoglycan and an outer membrane consisting of molecules of phospholipids, lipopolysaccharides (LPS), lipoproteins and surface proteins. The lipopolysaccharide consists of lipid A and O polysaccharide.

The peptidoglycan portion of the Gram-negative cell wall is generally 2-3 nanometers (nm) thick and contains just 2-3 layers of peptidoglycan (Figure 1C). Chemically, only 10 to 20% of the Gram-negative cell wall is peptidoglycan.

The outer membrane of the Gram-negative cell wall appears as a lipid bilayer about 7 nm thick. It is composed of phospholipids, lipoproteins, lipopolysaccharides (LPS), and proteins. Phospholipids are located mainly in the inner layer of the outer membrane, as are the lipoproteins that connect the outer membrane to the peptidoglycan (Figure 1A and 1B). The lipopolysaccharides, located in the outer layer of the outer membrane, consist of a lipid portion called lipid A embedded in the membrane and a polysaccharide portion extending outward from the bacterial surface. The LPS portion of the outer membrane is also known as endotoxin.
In addition, pore-forming proteins called porins (Figure 1B) span the outer membrane. The porins function as channels for the entry and exit of solutes through the outer membrane of the Gram-negative cell wall. The outer membrane of the Gram-negative cell wall is studded with surface proteins that differ with the strain and species of the bacterium.

The periplasm is the gelatinous material between the outer membrane, the peptidoglycan, and the cytoplasmic membrane. This periplasmic space is about 15nm wide and contains a variety of hydrolytic enzymes for nutrient breakdown, periplasmic binding proteins for transport via the ATP-binding cassette (ABC) system, and chemoreceptors for chemotaxis (discussed under Bacterial Flagella later in this Unit).

### Functions of the Gram-Negative Cell Wall Components

The peptidoglycan in the Gram-negative cell wall prevents osmotic lysis. The outer membrane of the Gram-negative cell wall confers several functions. Like the cytoplasmic membrane, is semipermeable and acts as a coarse molecular sieve. Many small molecules may pass through due to pores running through the membrane. These pores are composed of proteins called porins (Figure 1B). Because of its semipermeable nature, the outer membrane helps retain certain enzymes and prevents some toxic substances, such as penicillin G and lysozyme, from entering.

The LPS from the outer membrane of the Gram-negative cell wall (Figure 1B) is thought to add strength to the outer membrane, in a manner similar to the glycopeptides and teichoic acids of the gram-positive cell wall. The outer membrane may also form vesicles that contain quorum signaling molecules, enzymes, toxins, virulence factors, and even antibiotic resistance genes. These vesicles can then fuse with the outer membrane of other Gram-negative bacteria enabling them to communicate, obtain virulence factors, pick up resistance genes, or deliver toxins to human cells.

The surface proteins in the bacterial peptidoglycan (Figure 1B), depending on the strain and species, carry out a variety of activities. Some surface proteins function as enzymes, and other proteins serve as adhesins. Adhesins enable the bacterium to adhere intimately to host cells and other surfaces in order to colonize those cells and resist flushing (Figure 2).
**Figure 2:** Bacterial Adhesins. Surface proteins called adhesins in the bacterial cell wall bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make intimate contact with the host cell, adhere, colonize, and resist flushing.

Flash animation showing a bacterium using adhesins to adhere to a host cell.

html5 version of animation for iPad showing a bacterium using adhesins to adhere to a host cell.

Flash animation showing a bacterium using adhesins to resist being flushed out of the urethra.

html5 version of animation for iPad showing a bacterium using adhesins to resist being flushed out of the urethra.

Flash animation showing a bacterium without adhesins being flushed out of the urethra.

html5 version of animation for iPad showing a bacterium without adhesins being flushed out of the urethra.

c. Many bacteria involved in infection have the ability to **co-opt the functions of host cells 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 to the benefit of the bacteria. They do this by producing secretion systems such as the type 3 secretion system that produces hollow, needle-like tubes called **injectisomes**. Certain bacteria, for example, inject **invasins** into the cytoplasm of the host cell that enable the bacterium to **enter that cell**.

Flash animation showing a bacterium secreting invasions in order to penetrate non-immune host cells.

html5 version of animation for iPad showing a bacterium secreting invasions in order to penetrate non-immune host cells.

The role of these cell wall surface proteins will be discussed in greater detail later in Unit 3 under Bacterial Pathogenicity.

For More Information: The Ability to Adhere to Host Cells from Unit 3

For More Information: The Ability to Invade Host Cells from Unit 3
4. The periplasm contains enzymes for nutrient breakdown as well as periplasmic binding proteins to facilitate the transfer of nutrients across the cytoplasmic membrane.

The Role of Gram-Negative Cell Wall Components to the Initiation of Body Defenses

The body has two immune systems: the innate immune system and the adaptive immune system. 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. 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. This is the immunity one develops throughout life.

Initiation of Innate Immunity

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. (Because all microbes, not just pathogenic microbes, possess PAMPS, pathogen-associated molecular patterns are sometime referred to as microbe-associated molecular patterns or MAMPs.)

LPS, porins, and fragments of peptidoglycan are PAMPs associated with the cell wall of Gram-negative bacteria. In addition, bacteria and other microorganisms also possess mannose-rich glycans (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar) that function as PAMPs. These mannose-rich glycans are common in microbial glycoproteins and glycolipids but rare in those of humans (Figure 3).

These PAMPS bind to pattern-recognition receptors or PRRs on a variety of defense cells of the body and triggers innate immune defenses such as inflammation, fever, and phagocytosis.

Inflammation is the first response to infection and injury and is critical to body defense. Basically, the inflammatory response is an attempt by the body to restore and maintain homeostasis after injury. Most of the body defense elements are located in the blood, and inflammation is the means by which body defense cells and body defense chemicals leave the blood and enter the tissue around an injured or infected site.

Body defense cells called macrophages, and dendritic cells have pattern recognition receptors such as toll-like receptors on their surface that are specific for the peptidoglycan fragments and LPS in the Gram-negative cell wall and/or to NODs.
in their cytoplasm that are specific for peptidoglycan fragments. The binding of these cell wall components to their corresponding pattern recognition receptors 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 and initiate inflammation and activate both the complement pathways and the coagulation pathway (Figure 4).

The LPS binds to a LPS-binding protein circulating in the blood and this complex, in turn, binds to a receptor molecule (CD14) found on the surface of body defense cells called macrophages. This is thought to promote the ability of the toll-like receptor pair TLR-4/TLR4 to respond to the LPS. The binding of these cell wall components to their corresponding pattern recognition receptors triggers 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 and initiate inflammation and activate both the complement pathways and the coagulation pathway (Figure 4).

Exercise: Think-Pair-Share Questions

A large number of Gram-negative Escherichia coli have entered the urinary tract of a patient.

1. Explain how the body is able to recognize these bacteria and eventually send phagocytes and defense molecules to the infected site.
2. How might this mechanism lead to the symptoms of the infection?

The LPS also activates the alternative complement pathway and the lectin pathway, innate defense pathways that play a variety of roles in body defense.

Innate immunity will be discussed in greater detail in Unit 5.
Initiation of Adaptive Immunity

Proteins and polysaccharides associated with the Gram-negative cell wall function as antigens and initiate adaptive immunity. An antigen is defined as a molecular shape that reacts with antibody molecules and with antigen receptors on lymphocytes. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they fit specific antigen receptors on our B-lymphocytes and T-lymphocytes, the cells that carry out adaptive immunity.

The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called epitopes. An epitope is typically a group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen, or 3-4 sugar residues branching off of a polysaccharide antigen. A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against.

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).

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.

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 greater detail in Unit 6.

For More Information: Review of antigens and epitopes from Unit 6

Significance of Gram-Negative Cell Wall Components to Bacterial Pathogenicity

The lipid A portion of the LPS portion in the outer membrane is also known as endotoxin. During severe systemic infections with large numbers of bacteria present, high levels of LPS are released resulting in excessive cytokine production by the macrophages and other cells and this, in turn, can harm the body (Figure 5).

For More Information: Endotoxin from Unit 3

Concept map for the Gram-negative cell wall.
Summary

1. Because of the nature of their cell wall, Gram-negative bacteria stain pink after Gram staining.
2. The Gram-negative cell wall consists of 2-3 interconnected layers of peptidoglycan surrounded by an outer membrane.
3. Peptidoglycan prevents osmotic lysis in the hypotonic environment in which most bacteria live.
4. The outer membrane is a semipermeable structure that contains pore-forming proteins called porins that allow nutrients to pass through the outer membrane.
5. Surface proteins embedded in the cell wall can function as adhesins, secretion systems, and enzymes.
6. The Gram-negative cell wall activates both the body’s innate immune defenses and its adaptive immune defenses.
7. The body activates innate immunity by recognizing molecules unique to microorganisms that are not associated with human cells called pathogen-associated molecular patterns or PAMPs. PAMPs bind to Pattern-recognition receptors (PRRs) on defense cells to trigger the production of inflammatory cytokines.
8. Inflammation is the means by which the body delivers defense cells and defense molecules to an infection site, however, excessive inflammation, can be harmful and even deadly to the body.
9. PAMPs associated with the Gram-negative cell wall include peptidoglycan monomers, lipopolysaccharide (LPS), porins, and mannose-rich sugar chains.
10. An antigen is a molecular shape that reacts with antigen receptors on lymphocytes to initiate an adaptive immune response.
11. Cell wall molecules can also trigger adaptive immunity such as the production of antibody molecules against bacterial cell wall antigens.

Questions

Study the material in this section and then write out the answers to these questions. Do not just click on the answers and write them out. This will not test your understanding of this tutorial.

1. State what color Gram-negative bacteria appear after the Gram stain procedure. (ans)
2. Describe the structure and appearance of a Gram-negative cell wall. (ans)
3. State the beneficial function to the bacterium of the following components of the gram-negative cell wall:
   a. peptidoglycan (ans)
   b. outer membrane (ans)
   c. adhesins (ans)
   d. invasins (ans)
4. Briefly describe how the LPS (endotoxin) of the Gram-negative cell wall can promote inflammation. (ans)
5. Define epitope. (ans)
6. When Gram-negative bacteria enter the blood and cause septicemia, most of the harm to the body is due to a massive inflammatory response. What might explain this? (ans)
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