2.3C: The Acid-Fast Cell Wall

Fundamental Statements for this Learning Object:

In this section on Prokaryotic Cell Anatomy we are looking at the various anatomical parts that make up a bacterium. As mentioned in the introduction to this section, a typical bacterium usually consists of:

- a cytoplasmic membrane surrounded by a peptidoglycan cell wall and maybe an outer membrane;
- a fluid cytoplasm containing a nuclear region (nucleoid) and numerous ribosomes; and
- often various external structures such as a glycocalyx, flagella, and pili.

There are three primary types of bacterial cell wall: Gram-positive, Gram-negative, and acid-fast. We will now look at the acid-fast cell wall.

Acid-fast bacteria stain poorly with the Gram stain procedure, appearing weakly Gram-positive or Gram-variable. They are usually characterized using the acid-fast staining procedure. As mentioned in the previous section on peptidoglycan, bacteria with an acid-fast cell wall resist decolorization with an acid-alcohol mixture during the acid-fast staining procedure, retain the initial dye carbol fuchsin and appear red (Figure \(\PageIndex{1; left}\)). Common acid-fast bacteria of medical importance include *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Mycobacterium avium-intracellulare* complex, and *Nocardia* species.
Structure and Composition of the Acid-Fast Cell Wall

Acid-fast bacteria are gram-positive, but in addition to peptidoglycan, the outer membrane or envelope of the acid-fast cell wall contains large amounts of glycolipids, especially mycolic acids that in the genus *Mycobacterium*, make up approximately 60% of the acid-fast cell wall (Figure \(\PageIndex{2}\)).

- **Layer 1**: The acid-fast cell wall of *Mycobacterium* has a thin, inner layer of peptidoglycan.
- **Layer 2**: The peptidoglycan layer is, in turn, linked to arabinogalactan (D-arabinose and D-galactose).
- **Layer 3**: The arabinogalactan is then linked to an outer membrane containing high-molecular weight mycolic acids. The arabinogalactan/mycolic acid layer is overlaid with a layer of polypeptides and mycolic acids consisting of free lipids, glycolipids, and peptidoglycolipids. Other glycolipids include lipoarabinomannan and phosphatidylinositol mannosides (PIM). Like the outer membrane of the gram-negative cell wall, porins are required to transport small hydrophilic molecules through the outer membrane of the acid-fast cell wall.
- **Layer 4**: The outer surface of the acid-fast cell wall is studded with surface proteins that differ with the strain and species of the bacterium.
- **Layer 5**: The periplasm is the gelatinous material between the peptidoglycan and the cytoplasmic membrane.
Structure of an Acid-Fast Cell Wall. In addition to peptidoglycan, the acid-fast cell wall of Mycobacterium contains a large amount of glycolipids, especially mycolic acids. The peptidoglycan layer is linked to arabinogalactan (D-arabinose and D-galactose) which is then linked to high-molecular weight mycolic acids. The arabinogalactan/mycolic acid layer is overlaid with a layer of polypeptides and mycolic acids consisting of free lipids, glycolipids, and peptidoglycolipids. Other glycolipids include lipoarabinomannan and phosphatidylinositol mannosides (PIM). Like the outer membrane of the gram-negative cell wall, porins are required to transport small hydrophilic molecules through the outer membrane of the acid-fast cell wall. Because of its unique cell wall, when it is stained by the acid-fast procedure, it will resist decolorization with acid-alcohol and stain red, the color of the initial stain, carbol fuchsin. With the exception of a very few other acid-fast bacteria such as Nocardia, all other bacteria will be decolorized and stain blue, the color of the methylene blue counterstain.

Functions of the Acid-Fast Cell Wall Components

- **Layer 1**: The peptidoglycan prevents osmotic lysis.
- **Layer 2**: The arabinogalactan layer is linked to both the peptidoglycan and to the mycolic acid outer membrane and probably provides additional strength to the cell wall.
- **Layer 3**: The mycolic acids and other glycolipids also impede the entry of chemicals causing the organisms to grow slowly and be more resistant to chemical agents and lysosomal components of phagocytes than most bacteria (Figure \(\PageIndex{2}\)). There are far fewer porins in the acid-fast cell wall compared to the gram-negative cell wall and the pores are much longer. This is thought to contribute significantly to the lower permeability of acid-fast bacteria.
- **Layer 4**: The surface proteins in the acid-fast cell wall, depending on the strain and species, carry out a variety of activities, including functioning as enzymes and serving as adhesins, which enable the bacterium to adhere intimately to host cells and other surfaces in order to colonize and resist flushing.
- **Layer 15**: The periplasm contains enzymes for nutrient breakdown.
Exercise: Think-Pair-Share Questions

*Mycobacterium tuberculosis* is a very slow growing bacterium with a generation time often measured in days to weeks. It is also resistant to the vast majority of antibiotics that are commonly effective against other bacteria and treatment is typically with a combination of drugs for up to 9 months.

Based on what we just learned, explain what might account for these two characteristics.

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### Significance of Acid-Fast Cell Wall Components to the Initiation of Body Defenses

The body has two immune systems: the innate immune system and the adaptive immune system.

1. 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.

2. 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.

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### 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. Pathogenic *Mycobacterium* species such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* release mycolic acid, arabinogalactan, and peptidoglycan fragments from their acid-fast cell wall. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometime referred to as microbe-associated molecular patterns or MAMPs.)

These PAMPS bind to pattern-recognition receptors or PRRs on a variety of defense cells of the body causing them to synthesize and secrete a variety of proteins called cytokines. These cytokines can, in turn promote innate immune defenses such as inflammation, phagocytosis, activation of the complement pathways, and activation of the coagulation pathway.

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 mycolic acids in the acid-fast 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 and TNF-alpha. The cytokines then bind to cytokine receptors on target cells and initiate inflammation and activate both the complement pathways and the coagulation pathway.

Innate immunity will be discussed in greater detail in Unit 5.

**Initiation of Adaptive Immunity**

Proteins and polysaccharides associated with the acid-fast 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.

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.

**Significance of Acid-Fast Cell Wall Components to Bacterial Pathogenicity**

Most of the damage in the lungs during tuberculosis is thought to be due to the inflammatory effects from excessive TNF-alpha production, along with the release of toxic lysosomal components of the macrophages trying to kill the *Mycobacterium tuberculosis*.

Highlighted Bacterium: *Mycobacterium tuberculosis*

Click on this link, read the description of *Mycobacterium tuberculosis*, and be able to match the bacterium with its description on an exam.
Antimicrobial Agents that Inhibit Acid-Fast Cell Wall Synthesis to Control *Mycobacterium* Species

INH (isoniazid) blocks the incorporation of mycolic acid into acid-fast cell walls while ethambutol interferes with the incorporation of arabinoglactan (Figure \(\PageIndex{2}\)). Both inhibit synthesis of the acid-fast cell wall. Pyrazinamide inhibits fatty acid synthesis in *Mycobacterium tuberculosis*.

Think-Pair-Share Questions

Look at the following transmission electron micrograph and Gram stain of the same bacterium.

1. Is this organism Gram-positive, Gram-negative, or acid-fast?
2. How can you tell? State all reasons.

Summary

1. Because of the nature of their cell wall, acid-fast bacteria stain red after acid-fast staining.
2. The genus *Mycobacterium* and the genus *Nocardia* are among the few bacteria possessing an acid-fast cell wall.
3. The acid-fast cell wall consists of a thin, inner layer of peptidoglycan linked to a layer of arabinogalactin, which in turn is linked to an outer membrane containing mycolic acids and overlaid with a variety of polypeptides and glycolipids.
4. Porins are required to transport small hydrophilic molecules through the outer membrane of the acid-fast cell wall.
5. The acid-fast cell wall activates both the body's innate immune defenses and its adaptive immune defenses.

6. 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.

7. 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.

8. PAMPs associated with the acid-fast cell wall include peptidoglycan monomers, arabinogalactin, and mycolic acids.

9. An antigen is a molecular shape that reacts with antigen receptors on lymphocytes to initiate an adaptive immune response.

10. Cell wall molecules can also trigger adaptive immunity such as the production of antibody molecules against bacterial cell wall antigens.

11. A few antimicrobial chemotherapeutic agents inhibit acid-fast cell wall synthesis

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 acid-fast bacteria appear after the acid-fast stain procedure. (ans)

2. Describe the structure and appearance of an acid-fast cell wall. (ans)

3. State the beneficial function to the bacterium of the following components of the acid-fast cell wall:
   a. peptidoglycan (ans)
   b. mycolic acid and other glycolipids (ans)
   c. porins (ans)

4. Mycobacterium tuberculosis is much more resistant to antibiotics and disinfectants than most other bacteria. It also grows much more slowly. Why might this be? (ans)

5. Multiple Choice Cell Wall Quiz (ans)

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