2.5A: Glycocalyx (Capsules) and Biofilms

Skills to Develop

1. State the chemical composition and 2 common functions of a bacterial glycocalyx.
2. Briefly describe the following steps in phagocytosis:
   a. unenhanced attachment
   b. enhanced attachment
   c. engulfment
   d. destruction
3. Briefly describe how a capsule might initially enable some bacteria to resist being phagocytosed by white blood cells.
4. Define biofilm and state at least 3 advantages of biofilm formation to bacteria.

Highlighted Bacterium

1. Read the description of *Streptococcus pneumoniae* and match the bacterium with the description of the organism and the infection it causes.

All bacteria secrete some sort of glycocalyx (Capsules and Slime Layers), an outer viscous covering of fibers extending from the bacterium (see Figure 1, Figure 2, and Figure 3). If it appears as an extensive, tightly bound accumulation of gelatinous material adhering to the cell wall, it is called a capsule as shown in the photomicrograph in Figure 2. If the glycocalyx appears unorganized and more loosely attached, it is referred to as a slime layer.

**Structure and Composition**

The glycocalyx is usually a viscous polysaccharide or polypeptide slime. Actual production of a glycocalyx often
depends on environmental conditions.

- A capsule stain of *Streptococcus lactis*.

**Functions and Significance to Bacterial Pathogenicity**

Although a number of functions have been associated with the glycocalyx, such as protecting bacteria against drying, trap nutrients, etc., for our purposes there are two very important functions. The glycocalyx enables certain bacteria to resist phagocytic engulfment by white blood cells in the body or protozoans in soil and water. The glycocalyx also enables some bacteria to adhere to environmental surfaces (rocks, root hairs, teeth, etc.), colonize, and resist flushing.

1. **Preview of the Steps in Phagocytosis**

As will be seen in Unit 5, there are several steps involved in phagocytosis.

a. Attachment

First the surface of the microbe must be attached to the cytoplasmic membrane of the phagocyte. Attachment of microorganisms is necessary for ingestion and may be unenhanced or enhanced.

- 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 glycoprotein known as endocytic pattern-recognition receptors on the surface of the phagocytes (see Figure 4).

Flash animation illustrating the function of endocytic pattern-recognition receptors on phagocytes.

For More Information: Pathogen-Associated Molecular Patterns (PAMPs) from Unit 5

- Enhanced attachment is the attachment of microbes to phagocytes by way of an antibody molecule called IgG or proteins produced during the complement pathways called C3b and C4b (see Figure 5). Molecules such as IgG and C3b that promote enhanced attachment are called opsonins and the process is called opsonization. Enhanced attachment is much more specific and efficient than unenhanced.

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

Following attachment, polymerization and then depolymerization of actin filaments send pseudopods out to engulf the microbe (see Figure 6) and place it in a vesicle called a phagosome (see Figure 7).

Movie of a bacterium being engulfed by a neutrophil.
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You Tube Movie illustrating bacterial phagocytosis by a neutrophil.

You Tube Movie illustrating a neutrophil phagocytosing MRSA

YouTube movie showing phagocytosis of yeast by a white blood cell.

You Tube animation summarizing phagocytosis by a macrophage.

c. Destruction

Finally, lysosomes, containing digestive enzymes and microbicidal chemicals, fuse with the phagosome containing the
ingested microbe and the microbe is destroyed (see Figure 8).

Role of the Glycocalyx in Resisting Phagocytosis

Capsules enable bacteria to resist phagocytosis. For example, capsules can resist unenhanced attachment by preventing the glycoprotein receptors on phagocytes from recognizing the bacterial cell wall components and mannose-containing carbohydrates (see Figure 10). Also, 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 9). This will be discussed in greater detail later in Unit 3 under Bacterial Pathogenesis.

Examples of bacteria that use their capsule to resist phagocytic engulfment include Strep-toccus pneumoniae, Haemophilus influenzae type b, Neisseria meningitidis, Bacillus anthracis, and Bordetella pertussis.

- Encapsulated rod-shaped bacteria in an infected gall bladder.

For More Information: The Ability to Resist Phagocytic Engulfment from Unit 3

The body's immune defenses, however, can eventually get around the capsule by producing opsonizing antibodies (IgG) against the capsule. The antibody then sticks the capsule to the phagocyte. In vaccines against pneumococcal pneumonia and Haemophilus influenzae type b, it is capsular polysaccharide that is given as the antigen in order to stimulate the body to make opsonizing antibodies against the encapsulated bacterium.

Highlighted Bacterium: Streptococcus pneumoniae

Click on this link, read the description of Streptococcus pneumoniae, and be able to match the bacterium with its description on an exam.

Exercise: Think-Pair-Share Questions

*Streptococcus pneumoniae*, an encapsulated bacterium, enters the respiratory tract of a young child for the first time while that child has influenza. The child subsequently develops pneumococcal pneumonia, is treated with antibiotics, and recovers.

1. Normally when bacteria first enter the body, the innate immune defenses bind PAMPs on the bacterial cell wall to endocytic PRRs on the body's phagocytes and the organism is phagocytosed. Explain why the child's innate phagocytic defense was unable to remove the *S. pneumoniae*.

2. The pneumococcal conjugate vaccine, PCV13 or Prevnar 13® is currently recommended for all children under 5 years of age. Why might prior vaccination with this vaccine have enabled the child to to remove the *S. pneumoniae* via phagocytosis?

3. **Role of the Glycocalyx in Adhering to and Colonizing Environmental Surfaces**

The glycocalyx also enables some bacteria to adhere to environmental surfaces (rocks, root hairs, teeth, etc.), colonize, and resist flushing. For example, many normal flora bacteria produce a capsular polysaccharide matrix or glycocalyx to form a biofilm on host tissue (see Figure 3) as discussed below.

**Significance of the glycocalyx in the Initiation of Body Defense**

**Initiation of Adaptive Immunity**

Polysaccharides or polypeptides associated with the bacterial glycocalyx or capsule 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 capsular antigens can stick bacteria to phagocytes, a process called opsonization.

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.

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**Biofilms**

Many pathogenic bacteria, as well as normal flora and many environmental bacteria, form complex bacterial communities as biofilms. Biofilms are groups of bacteria attached to a surface and enclosed in a common secreted adhesive matrix, typically polysaccharide in nature. Bacteria in biofilms are often able to communicate with one another by a process called quorum sensing (discussed later in Unit 2) and are able to interact with and adapt to their environment as a population of bacteria rather than as individual bacteria. By living as a community of bacteria as a biofilm, these bacteria are better able to:

- resist attack by antibiotics;
- trap nutrients for bacterial growth and remain in a favorable niche;
- adhere to environmental surfaces and resist flushing;
- live in close association and communicate with other bacteria in the biofilm; and
- resist phagocytosis and attack by the body's complement pathways.

Biofilms are, therefore, functional, interacting, and growing bacterial communities. Biofilms even contain their own water channels for delivering water and nutrients throughout the biofilm community.

- Electron micrograph of a biofilm of *Haemophilus influenzae* from Biomedcentral.com
- Photomicrograph of a biofilm with water channels from Centers for Disease Control and Prevention Rodney M. Donlan: "Biofilms: Microbial Life on Surfaces"
- Biofilm of *Pseudomonas aeruginosa* from the Ausubel Lab, Department of Molecular Biology, Massachusetts General Hospital

To initiate biofilm formation, planktonic bacteria (free individual bacteria not in a biofilm) contact an environmental surface through their motility or by random collision. These planktonic bacteria then attach to that surface using pili or cell wall adhesins. This attachment then signals the expression of genes involved in quorum sensing and, ultimately, biofilm formation. As the biofilm matrix is secreted, motile bacteria lose their flagella and become nonmotile.

Planktonic *Pseudomonas aeruginosa*, for example, uses its polar flagellum to move through water or mucus and make contact with a solid surface such as the body's mucous membranes. It then can use pili and cell wall adhesins to attach to the epithelial cells of the mucous membrane. Attachment activates signaling and quorum sensing genes to eventually enable the population of *P. aeruginosa* to start synthesizing a polysaccharide biofilm composed of alginate. As the biofilm grows, the bacteria lose their flagella to become nonmotile and secrete a variety of enzymes that enable the
population to obtain nutrients from the host cells. Eventually the biofilm mushrooms up and develops water channels to deliver water and nutrients to all the bacteria within the biofilm. As the biofilm begins to get too crowded with bacteria, quorum sensing enables some of the *Pseudomonas* to again produce flagella, escape the biofilm, and colonize a new location (See Figs. 11A-11G).

*Streptococcus mutans*, and *Streptococcus sobrinus*, two bacteria implicated in initiating dental caries, break down sucrose into glucose and fructose. *Streptococcus mutans* can uses an enzyme called dextranseucrase to convert sucrose into a sticky polysaccharide called dextran that forms a biofilm enabling the bacteria to adhere to the enamel of the tooth and form plaque. This will be discussed in greater detail later in Unit 2 under Bacterial Pathogenicity. *S. mutans* and *S. sobrinus* also ferment glucose in order to produce energy. The fermentation of glucose results in the production of lactic acid that is released onto the surface of the tooth and initiates decay.

- Scanning electron micrograph of *Streptococcus* growing in the enamel of a tooth.© Lloyd Simonson, author. Licensed for use, ASM MicrobeLibrary.
- Scanning electron micrograph of dental plaque.© H. Busscher, H. van der Mei, W. Jongebloed, R Bos, authors. Licensed for use, ASM MicrobeLibrary.
- Scanning electron micrograph of *Staphylococcus aureus* forming a biofilm in an indwelling catheter courtesy of CDC.
- Biofilm of *Staphylococcus aureus* from Montana State University

A number of biofilm-forming bacteria, such as uropathogenic *Escherichia coli* (UPEC), enterohemorrhagic *E. coli* (EHEC), *Citrobacter* species, *Salmonella* species, and *Mycobacterium tuberculosis* are able to produce amyloid fibers that can play a role in such processes as attachment to host cells, invasion of host cells, and biofilm formation. *Curli* is an example of such an amyloid fiber produced by UPEC and *Salmonella*.

Many chronic and difficult-to-treat infections are caused by bacteria in biofilms. Within biofilms, bacteria grow more slowly, exhibit different gene expression than free planktonic bacteria, and are more resistant to antimicrobial agents such as antibiotics because of the reduced ability of these chemicals to penetrate the dense biofilms matrix. Biofilms have been implicated in tuberculosis, kidney stones, *Staphylococcus* infections, Legionnaires’ disease, and periodontal disease. It is further estimated that as many as 10 million people a year in the US may develop biofilm-associated infections as a result of invasive medical procedures and surgical implants.
Summary

1. All bacteria secrete some sort of glycocalyx, an outer viscous covering of fibers extending from the bacterium.
2. An extensive, tightly bound glycocalyx adhering to the cell wall is called a capsule.
3. Phagocytosis involves several distinct steps including attachment of the microbe to the phagocyte through unenhanced or enhanced attachment, ingestion of the microbe and its placement into a phagosome, and the destruction of the microbe after fusion of lysosomes with the phagosome.
4. Capsules enable bacteria to resist unenhanced attachment by covering up bacterial PAMPs so they are unable to bind to endocytic pattern-recognition receptors.
5. The glycocalyx also enables some bacteria to adhere to environmental surfaces, colonize, and resist flushing.
6. The body's adaptive immune defenses can eventually overcome bacterial capsules by producing opsonizing antibodies (IgG) against the capsule that are able to stick the capsule to the phagocyte.
7. Biofilms are groups of bacteria attached to a surface and enclosed in a common secreted adhesive matrix and are functional, interacting, and growing bacterial communities.
8. Most bacteria in nature exist as biofilm populations.
9. Many chronic and difficult-to-treat infections are caused by bacteria in biofilms.

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 two common functions associated with the bacterial glycocalyx. (ans)
2. Briefly describe how a bacterial capsule might block phagocytosis. (ans)
3. State three possible functions associated with a bacterial biofilm. (ans)
4. Multiple Choice (ans)

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