Unit 3:
Bacterial Pathogenesis

Bacterial Pathogenesis: Virulence Factors that Promote Bacterial Colonization; Virulence Factors that Harm the Body.

An overview of microbial pathogenicity.
AN OVERVIEW OF MICROBIAL PATHOGENESIS

An Overview of Microbial Pathogenesis

Fundamental Statements for this Softchalk Lesson:

1. Only a relatively few bacteria cause human disease.
2. The complex mutually beneficial symbiotic relationship between humans and their natural microbes is critical to good health.
3. An infection is when a microorganism has established itself in a host - has colonized that host - whether not it causing harm or imparting damage.
4. A disease is where there is impairment to host function as a result of damage or injury.
5. Etiology refers to the causes of diseases or pathologies; in terms of infectious disease, the etiologic agent is the microorganism causing that disease.
6. A sign is an objective indication of some medical fact or characteristic that may be detected by a healthcare professional during a physical examination; a symptom is a condition experienced and reported by the patient.
7.The reservoir of an infectious agent is the habitat in which that microbe normally lives, grows, and multiplies.
8. Transmission of microorganisms by direct contact refers to transfer by such means as skin-to-skin contact, kissing, and sexual intercourse.
9. Transmission of microorganisms by direct droplet contact refers to transfer by aerosols produced by sneezing and coughing.
10. Transmission of microorganisms by indirect contact refers to transfer by suspended air particles, inanimate objects, or vectors (ticks, mosquitoes, fleas).
11. The manner in which a pathogen enters a susceptible host is referred to as its portal of entry; the manner in which it leaves its host is its portal of exit.
12. If relatively few bacteria enter the body then the body's natural defenses against infection have a much better chance of removing them before they can colonize, multiply, and cause harm; if a large number of bacteria enter then the body's defenses may not be as successful.
13. A person with good innate and adaptive immune defenses will be much more successful in removing potentially harmful bacteria than a person that is immunocompromised.
14. Bacterial virulence factors influence a bacterium's ability to cause infectious disease. These include virulence factors that enable bacteria to colonize the host as well as those that harm or damage the host.

Common Course Objectives

1. Describe the role of normal flora in health and in disease.
2. Explain what a reservoir is and how it can contribute to disease.
3. Explain what is meant by zoonosis and give major examples.
4. Recall the factors that influence disease severity.
5. Compare and contrast infection and disease.
6. Explain the significance of portals of entry and exit in disease.
7. Describe the progression of symptoms in a disease and recall the difference between a symptom and a sign.
8. Explain how diseases can be transmitted.
9. Determine the significance of emerging pathogens.

Detailed Learning Objectives

1. Define the following:

    a*. pathogenicity

    b*. virulence

    c*. virulence factors

    d*. infection

    e*. disease

    f. etiologic agent

    g. reservoir

    h. zoonosis

    i. vector

    j. portal of entry and portal of exit

2. Compare and contrast sign and symptom.

3. List 4 requirements for a microorganism to cause infectious disease.

4. Contrast and give examples of direct and indirect transmission of microorganisms.

5. Even though a microorganism may be considered pathogenic, it still may not be able to cause disease upon entering the body. Discuss why.

    (*) = Common theme throughout the course

    (**) = More depth and common theme

An Overview of Microbial Pathogenesis

1. Infection versus Disease

In this course we are looking at various fundamental concepts of microbiology, with particular emphasis on their relationships to human health. The **overall goal** is to better understand the total picture of infectious diseases in terms of host-infectious agent interaction.

Bacteria are found in almost every environment. Only a **relatively few bacteria cause human disease** and many benefit humans. For example, many are important decomposers that assure the flow and recycling of nutrients through ecosystems. Others have important industrial and pharmaceutical uses.

While the typical human body contains an estimated 10 trillion human cells, it also contains over 100 trillion bacteria and other microbes. **The complex mutually beneficial symbiotic relationship between humans and their natural microbes is critical to good health**. It is now recognized that **the millions of genes associated with the normal flora or microbiota of the human body -especially in the intestinal tract - aid in the digestion of many foods, the regulation of multiple host metabolic pathways, and the regulation the body's immune defenses**. These collective microbial genes are referred to as the **human microbiome**. There are currently an estimated 3, 000,000 - 5,000,000 genes from over 1000 species that

constitute the human microbiome compared to the approximately 23,000 genes that make up the human genome. Some of these same normal micribiota, however, can also cause opportunistic infections when they get into parts of the body where they do not normally live or when the body becomes immunosuppressed.

However, in this section we are going to concentrate on bacteria that are potentially harmful to humans and try to **understand what factors influence their ability to cause disease**.

Pathogenicity and virulence are terms that refer to an organism's ability to cause disease. **Pathogenicity is the ability of a microbe to cause disease and inflict damage upon its host**, whereas **virulence is the degree of pathogenicity within a group or species of microbes** as indicated by case fatality rates and/or the ability of the organism to invade the tissues of the host. The pathogenicity of an organism, that is its ability to cause disease, is **determined by its virulence factor**s.

As learned earlier under Bacterial Genetics, most of the virulence factors that enable bacteria to colonize the body and/or harm the body are the products of quorum sensing genes. **Many bacteria use quorum sensing to sense their own population density, communicate with each other by way of secreted chemical factors, and behave as a population rather than as individual bacteria**. This plays an important role in pathogenicity and survival for many bacteria.

The **genomes of pathogenic bacteria**, when compared with those of similar nonpathogenic species or strains, **often show extra genes coding for virulence factors**, that is, **molecules expressed and secreted by the bacterium that enable them to colonize the host, evade or inhibit the immune responses of the host, enter into or out of a host cell, and/or obtain nutrition from the host**. These include virulence factors such as capsules, adhesins, type 3 secretion systems, invasins, and toxins.

We also learned that **most genes coding for virulence factors in bacteria are located in pathogenicity islands or PAIs** and are usually acquired by horizontal gene transfer. These PAIs may be located in the bacterial chromosome, in plasmids, or even in bacteriophage genomes that have entered the bacterium. The genomes of most pathogenic bacteria typically contain multiple PAIs that can account for up to 10 - 20% of the bacterium's genome. PAIs carry genes such as transpoases integrases, or insertion sequences that **enable them to insert into host bacterial DNA**. Transfer RNA (tRNA) genes are often the target site for integration of PAIs. Conjugative plasmids are the most frequent means of transfer of PAIs from one bacterium to another and **the transfer of PAIs can then confer virulence to a previously nonpathogenic bacterium**.

For more information: Review of quorum sensing and pathogenicity islands

An infection is **when a microorganism has established itself in a host - has colonized that host - whether not it causing harm or imparting damage**. A disease, on the other hand, **is where there is impairment to host function as a result of damage or injury**. For example, the microbes that constitute the body's normal flora or microbiota have infected the body, but they seldom cause disease unless they invade a part of the body where they do not normally reside and/or the host becomes immunocompromised. In medicine, the term etiology refers to the causes of diseases or pathologies. In terms of infectious disease, the etiologic agent **is the microorganism causing that disease**.

The terms signs and symptoms are often used when diagnosing disease. A sign **is an objective indication of some medical fact or characteristic that may be detected by a healthcare professional during a physical examination**. They include such objective indications as blood pressure, respiration, rate, pulse, and temperature. A symptom is **a condition experienced and reported by the patient**.

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Concept Map for an Overview of Microbial Pathogenicity

2. Causing Infectious Disease

To cause disease, a microorganism must:

a. Maintain a reservoir before and after infection.

The reservoir of an infectious agent is **the habitat in which that microbe normally lives, grows, and multiplies**. Reservoirs can include **humans, animals, and the environment**. Many common human infectious diseases have human reservoirs and are transferred person-to-person without intermediaries. Examples include sexually transmitted diseases, measles, most respiratory pathogens, and strep throat. Some infections are transmitted from an animal to a human in which case the infection is called a zoonosis. Examples include rabies, plague, and much salmonellosis. Plants, soil, and water in the environment are also reservoirs for some infectious agents such as histoplasmosis, coccidioidomycosis, and Legionnaires disease.

b. Leave the reservoir and gain access to the new host.

The microorganism must leave its reservoir or host through what is called a **portal of exit** and be transmitted to a new host. For example, the portal of exit for respiratory infections is typically the mouth or nose; for gastrointestinal infections, the feces. Modes of transmission include:

1. **Direct contact**, as through skin-to-skin contact, kissing, and sexual intercourse. Examples include some *Staphylococcus aureus* infections, infectious mononucleosis, and gonorrhea.

2. **Direct droplet contact**, as in the case of aerosols produced by sneezing and coughing. Examples include meningococcal infections and pertussis (whooping cough).

3. **Indirect transmission** of an infectious agent from a reservoir to a host by suspended air particles, inanimate objects, or vectors.

   a. **Airborne transmission** occurs when infectious agents are carried by dust or droplets suspended in air. Some respiratory infections can be transmitted this way although most are transmitted by contact with infectious mucus.

   b. **Inanimate objects** include water, food, blood, and fomites (inanimate objects such as toys, handkerchiefs, bedding, or clothing). Examples include cholera, salmonellosis, listeriosis, viral hepatitis).

   c. Vectors such as ticks, mosquitoes, and fleas. Examples include Lyme's disease, malaria, and typhus fever.

The manner in which a pathogen enters a susceptible host is referred to as its **portal of entry**. For example, the portal of entry for most respiratory infections is the mouth or nose; for gastrointestinal infections, the mouth. The **portal of entry must provide access to tissues with the correct physical and chemical environment** (an environment with the proper oxygen content, pH, nutrients, temperature, etc.) **in which the pathogen can multiply**.

c. Adhere to cells of the skin or mucosa of its new host and colonize the body.

Almost every part of the body has a mechanism for flushing microbes out of or off of the body, including the shedding of epithelial cells from the skin and mucous membranes, urination, defecation, coughing, and sneezing. Unless the microorganisms can replicate fast enough to replace those being flushed out, as in the case of much of the normal microbiota that colonize the lumen of the intestines, they need to adhere to the epithelial cells of the skin and mucous membranes. Also, this body environment must have the correct nutrients, the proper amount of oxygen or lack of oxygen, the right pH, and the right temperature to support the growth of that microorganism. Furthermore, since the body has excellent immune defense mechanisms, anything the microorganism can do to resist body defenses to some degree will also promote colonization.

d. Harm or damage the body.

As stated above, an infection is simply when a microorganism has established itself in a host. To cause disease, that microorganism (or toxin) must inflict damage to the host.

In this unit we are going to take up bacterial pathogenesis. Anything the bacterium does to aid in the requirements needed to cause infectious disease mentioned above will influence its ability to cause disease. Bacteria are able to carry out many of these requirements as a result of their virulence factors. We must keep in mind, however, that **whether or not a person actually contracts an infectious disease after exposure to a particular potentially pathogenic bacterium depends not only on the microorganism, but also on the number of bacteria that enter the body and the quality of the person's innate and adaptive immune defenses**. (Innate and adaptive immunity will be discussed in detail in later Units.)

For example, if **relatively few bacteria** enter the body then the body's natural defenses against infection have a much better chance of removing them before they can colonize, multiply, and cause harm. On the other hand, if a **large number of bacteria** enter then the body's defenses may not be as successful.

Likewise, a person with **good innate and adaptive immune defenses** will be much more successful in removing potentially harmful bacteria than a person that is immunocompromised. In fact **a person highly immunosuppressed**, such as a person taking immunosuppressive drugs to suppress transplant rejection, or a person with advancing HIV infection, or a person with other immunosuppressive disorders, becomes very susceptible to infections by microorganisms generally considered not very harmful to a healthy person with normal defenses.

However, in this unit we are going to look at **bacterial virulence factors** that can influence its ability to cause infectious disease. Virulence factors are molecules expressed and secreted by microorganisms that enable them to colonize the host, evade or inhibit the immune responses of the host, enter into or out of a host cell, and/or obtain nutrition from the host. These virulence factors will be divided into two categories:

A. Virulence factors that promote bacterial colonization of the host.

There are 6 factors we will eventually discuss in this unit that will promote bacterial colonization of humans:

1. The ability to use motility and other means to contact host cells and disseminate within a host
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells
4. The ability to compete for iron and other nutrients
5. The ability to resist innate immune defenses such as phagocytosis and complement
6. The ability to evade adaptive immune defenses

B. Virulence factors that damage the host.

There are 3 factors we will eventually discuss in this unit that can result in harm to humans:

1. The ability to produce cell wall components (pathogen-associated molecular patterns or PAMPS) that bind to host cells causing them to synthesize and secrete inflammatory cytokines and chemokines
2. The ability to produce harmful exotoxins
3. The ability to induce autoimmune responses

iBiology YouTube Lecture on Microbial Pathogenicity

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Concept Map for an Overview of Microbial Pathogenicity

Self Quiz for Overview of Microbial Pathogenesis

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Bacterial use of motility to contacy host cells and disseminate within a host.
VIRULENCE FACTORS THAT PROMOTE BACTERIAL COLONIZATION OF THE HOST

1. The Ability to Use Motility or Other Means to Contact Host Cells and Disseminate Within a Host

Fundamental Statements for this Softchalk Lesson:

1. Bacteria have to make physical contact with host cells before they can adhere to those cells and resist being flushed out of the body.
***2. Motile bacteria can use their flagella and chemotaxis to swim through mucus towards mucosal epithelial cells*.**
3. ***Because of their thinness, their internal flagella (axial filaments), their corkscrew shape, and their motility, certain spirochetes are more readily able enter lymph vessels and blood vessels and spread to other body sites.***
4. Many bacteria produce enzymes that degrade the extracellular matrix proteins that surround cells and tissues and help to localize infection, making it easier for those bacteria to spread within the body.
5. Some bacteria produce toxins that induce diarrhea in the host enabling the pathogen to more readily leave one host and enter new hosts through the fecal-oral route.

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that may harm the host and give relevant examples.

Detailed Learning Objectives

1*. State why it might be of an advantage for a bacterium trying to colonize the bladder or the intestines to be motile.

2**. Describe specifically how certain bacteria are able to use motility to contact host cells and state how this can promote colonization.

3*. Briefly describe why being extremely thin and being motile by means of axial filaments may be an advantage to pathogenic spirochetes.

4. Give one example of how a nonmotile bacterium may be able to better disseminate within a host.

5. Give a brief description of how a bacterium may use toxins to better disseminate from one host to another.

(*) = Common theme throughout the course

(**) = More depth and common theme

Highlighted Bacterium

1. Read the description of *Helicobacter pylori* and match the bacterium with the description of the organism and the infection it causes.

In this Unit on Bacterial Pathogenesis we are looking at **virulence factors that promote bacterial colonization of the host**. The following are virulence factors that promote bacterial colonization of the host .

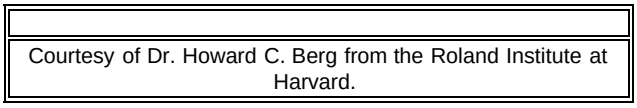
1. The ability to use motility and other means to contact host cells and disseminate within a host.
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells.
4. The ability to compete for iron and other nutrients.
5. The ability to resist innate immune defenses such as phagocytosis and complement.
6. The ability to evade adaptive immune defenses.

We will now look at virulence factors that enable bacteria to contact host cells.

The Ability to Use Motility or Other Means to Contact Host Cells and Disseminate Within a Host

The mucosal surfaces of the respiratory tract, the intestinal tract, and the genitourinary tract constantly flush bacteria away in order to prevent colonization of host mucous membranes. **Motile bacteria can use their motility and chemotaxis to swim through mucus towards mucosal epithelial cells**. Many bacteria that can colonize the mucous membranes of the bladder and the intestines, in fact, are motile. Motility probably helps these bacteria move through the mucus between the mucin strands or in places where the mucus is less viscous. Examples of motile opportunists and pathogens include *Helicobacter pylori, Salmonella* species, *Escherichia coli, Pseudomonas aeruginosa*, and *Vibrio cholerae*. Once bacteria contact host cells they can subsequently attach, and colonize. (Attachment will be discussed in the next section.)

Flash animation showing a motile bacterium contacting a host cell by swimming through the mucus.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a motile bacterium contacting a host cell by swimming through the mucus.
The mucosal surfaces of the bladder and the intestines constantly flush bacteria away in order to prevent colonization. Motile bacteria that can swim chemotactically toward mucosal surfaces may have a better chance to make contact with the mucous membranes, attach, and colonize. Many bacteria that can colonize the mucous membranes of the bladder and the intestines are motile. Motility probably helps these bacteria move through the mucus in places where it is less viscous.



Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

For example, *Helicobacter pylori*, the bacterium that causes most gastric and duodenal ulcers, **produces urease, an enzyme that breaks down urea into ammonia and bicarbonate, basic compounds that neutralize the hydrochloric acid in the stomach**. In addition, **the urease is thought to alter the**

proteins in the mucus changing it from a solid gel to a thinner fluid that the bacteria are able to swim through by way of their flagella. Using motility and chemotaxis, the bacteria move away from the concentrated acid (a repellent) in the lumen of the stomach towards decreasing acid concentration on the epithelium and in the gastric glands of the stomach. At the same time it also uses chemotaxis to swim towards urea (an attractant) being produced by stomach cells. *H. pylori* subsequently use adhesins to adhere to the epithelial cells of the mucous membranes and to the cells that line the gastric glands. To further help protect the bacterium from the acid, *H. pylori* produces an acid-inhibitory protein that blocks acid secretion by surrounding parietal cells in the stomach. Bacterial toxins then lead to excessive production of cytokines and chemokines, as well as mucinase and phospholipase that damage the gastric mucosa. The cytokines and chemokines, in turn, result in a massive inflammatory response. Neutrophils leave the capillaries, accumulate at the area of infection, and discharge their lysosomes for extracellular killing. This not only kills the bacteria, it also destroys the mucus-secreting mucous membranes of the stomach. *H. pylori* also **secretes toxins such as vacuolating cytotoxin A (VacA) that damages host cells causing them to release nutrients** to feed the microcolonies of *H. pylori*. Without this protective mucus layer, gastric acid causes ulceration of the stomach. This, in turn, leads to either gastritis or gastric and duodenal ulcers.

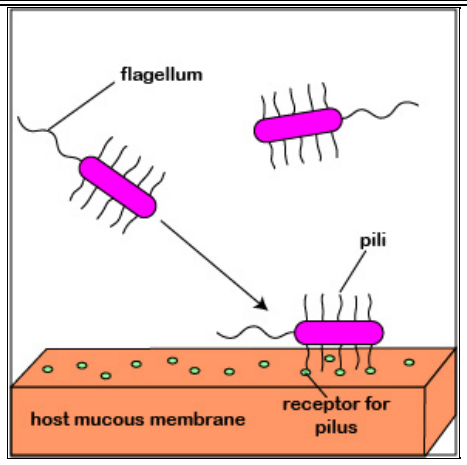
Flash animation showing induction of stomach and intestinal ulcers by *Helicobacter pylori*.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing induction of stomach and intestinal ulcers by *Helicobacter pylori*.
Helicobacter pylori, by means of its flagella, is able to swim through the mucus layer of the stomach or intestines and adhere to the epithelial cells of the mucous membranes. Here the pH is near neutral. To also help protect the bacterium from the acid, *H. pylori* produces an acid-inhibitory protein that blocks acid secretion by surrounding parietal cells in the stomach. The bacterium then releases toxins that lead to excessive production of cytokines and chemokines, as well as mucinase and phospholipase that damage the gastric or intestinal mucosa. The cytokines and chemokines, in turn, result in a massive inflammatory response. Neutrophils leave the capillaries, accumulate at the area of infection, and discharge their lysosomes for extracellular killing. This not only kills the bacteria, it also destroys the mucus-secreting mucous membranes of the stomach. Without this protective layer, gastric acid causes ulceration of the stomach or intestines. (Note that *H. pylori* is actually a spiral-shaped bacteria with a lophotrichous arrangement of flagella but showing this in the animation is beyond my technical abilities.)

Highlighted Bacterium: *Helicobacter pylori*

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

Planktonic *Pseudomonas aeruginosa* uses its polar flagellum to move through water or mucus and make contact with a solid surface such as the body's mucous membranes **(see Figs. 6A)**. 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.

Fig. 1: Development of a Biofilm by *Pseudomonas aeruginosa*; Role of Flagella



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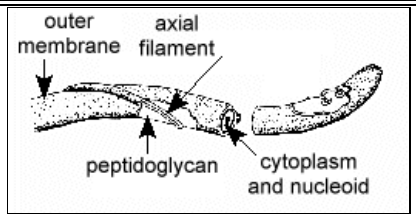
Planktonic *Pseudomonas aeruginosa* use their polar flagella and chemotaxis to swim towards host mucous membranes. Pili then bind to host cell receptors for initial but reversible bacterial attachment.

For more information: Prokaryotic flagella

Because of their thinness, their internal flagella (axial filaments), their corkscrew shape, and their motility **(see Fig. 2)**, **spirochetes** are more readily able to **penetrate** host mucous membranes, skin abrasions, etc., and **enter the body**. Motility and penetration may also enable the spirochetes to penetrate deeper in tissue and **enter the lymphatics and bloodstream** and disseminate to other body sites. Spirochetes that infect humans include *Treponema pallidum*, *Leptospira*, and *Borrelia burgdorferi*.

Fig. 2: Illustration of Spirochete Axial Filaments



Flash animation showing spirochetes using motility to enter a blood vessel.

html5 version of animation for iPAD showing spirochetes using motility to enter a blood vessel.

A combination of motility and invasins appears to helps *Borrelia bergdorferi* and *Treponema pallidum* to invade and exit blood vessels by passing between and through endothelial cells. This enables these spirochetes to disseminate to other locations in the body. One tip of the spirochete attaches to the host cell and some form of invasin apparently causes the host cell to release digestive enzymes that enable the spirochete with its corkscrewing motility to penetrate the host cell membrane.

Along a different line, many bacteria produce enzymes such as elastases and proteases that degrade the extracellular matrix proteins that surround cells and tissues and make it easier for those bacteria to disseminate within the body. For example, *Streptococcus pyogenes* produces streptokinase that lyses the fibrin clots produced by the body in order to localize the infection. It also produces DNase that degrades cell-free DNA found in pus and reduces the viscosity of the pus. Both of these enzymes facilitate spread of the bacterium from the localized site to new tissue.

Staphylococcus aureus, on the other hand, produces surface adhesins that bind to extracellular matrix proteins and polysaccharides surrounding host cell tissue, including fibronectin, collagen, laminin, hyaluronic acid, and elastin. *S. aureus* proteases and hyaluronidase then dissolve these components of the extracellular matrix providing food for the bacteria and enabling the bacteria to spread.

Finally, as will be seen later in this unit under toxins, some bacteria produce toxins that induce diarrhea in the host. Diarrhea is also a part of our innate immunity to flush harmful microbes and toxins out of the intestines. On one hand, diarrhea is an advantage to the body because it flushes out harmful microbes and toxins. On the other hand, it is beneficial for the bacterium inducing the diarrhea because it also flushes out a good deal of the normal flora of the intestines and this reduces the competition for nutrients between normal flora and pathogens. In addition, diarrhea enables the pathogen to more readily leave one host and enter new hosts through the fecal-oral route.

Concept map for Bacterial Colonization of Host Cell: Using Motility to Contact Host Cell.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Vibrio cholerae*
- *Treponema pallidum*
- *Leptospira*
- *Borrelia burgdorferi*
- *Helicobacter pylori*

Self Check for The Ability to Use Motility or Other Means to Contact Host Cells and Disseminate Within a Host

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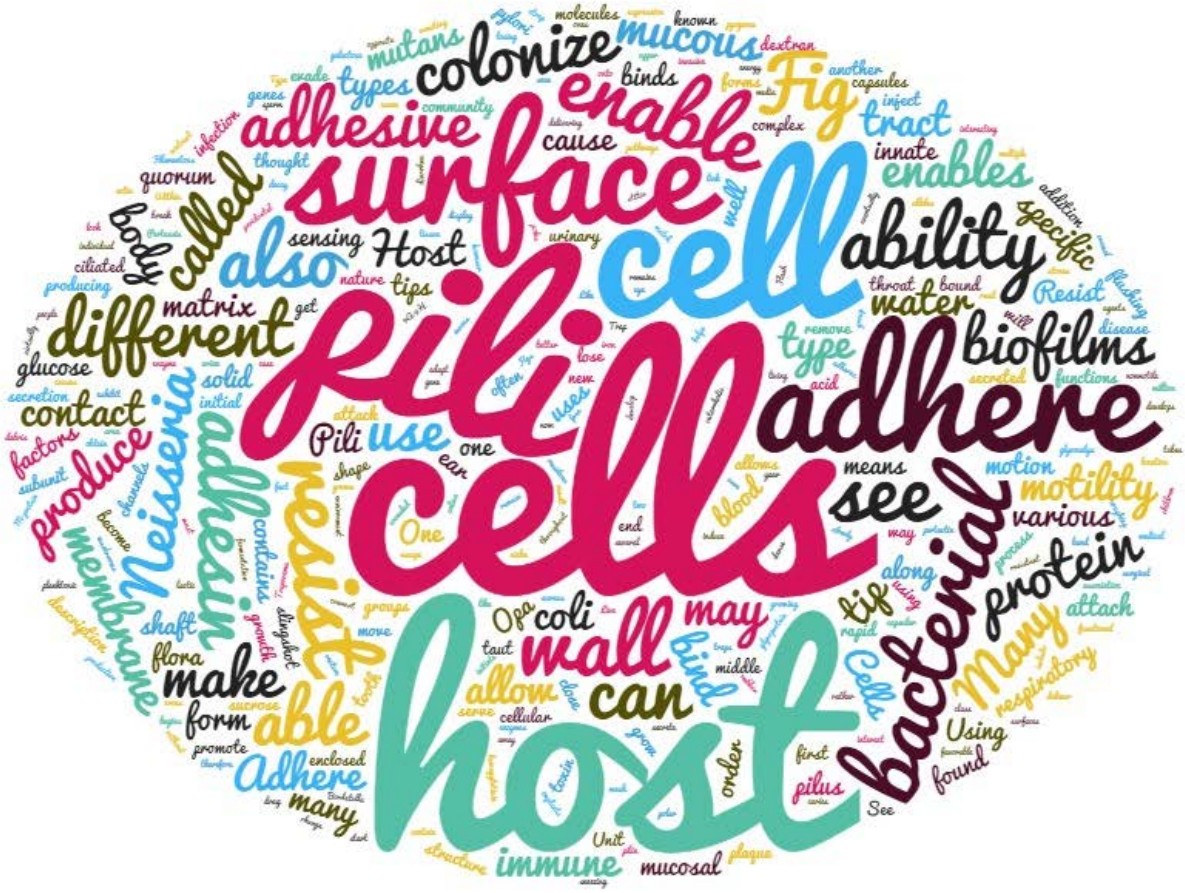
How bacteria adhere to host cells in order to colonize and resist physical removal.:
VIRULENCE FACTORS THAT PROMOTE BACTERIAL COLONIZATION OF THE HOST

2. The Ability to Adhere to Host Cells and Resist Physical Removal



Fundamental Statements for this Softchalk Lesson:

1. One of the body's innate immune defenses is the ability to physically remove bacteria from the body.
2. Bacteria may resist physical removal by producing pili, cell wall adhesin proteins, and/or biofilm-producing capsules that enable bacteria to adhere to host cells.
3. At the end of the shaft of a bacterial pilus is an adhesive tip structure having a shape corresponding to that of specific receptor on a host cell for initial attachment. Bacteria can typically make a variety of different adhesive tips enabling them to attach to different host cell receptors.
4. Cell wall adhesins are surface proteins found in the cell wall of various bacteria that bind tightly to specific receptor molecules on the surface of host cells. Bacteria can typically make a variety of different cell wall adhesins enabling them to attach to different host cell receptors.
5. Biofilms are groups of bacteria attached to a surface and enclosed in a common secreted adhesive matrix, typically polysaccharide in nature. Many pathogenic bacteria, as well as normal flora and many environmental bacteria, form complex bacterial communities as biofilms.
6. Many chronic and difficult-to-treat infections are caused by bacteria in biofilms.

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that promote microbial colonization of a host and give relevant examples.

Detailed Learning Objectives

1**. Briefly **describe** 3 different mechanisms by which bacteria can adhere to host cells and colonize and state how this can promote colonization.

2. State an advantage for bacteria in being able to switch the adhesive tips of their pili.

3*. Define biofilm and state at least 3 benefits associated with bacteria living as a community within a biofilm.

(*) = Common theme throughout the course

(**) = More depth and common theme

Highlighted Bacterium

1. Read the description of *Neisseria meningitidis* and match the bacterium with the description of the organism and the infection it causes.

TPS Questions

In this Unit on Bacterial Pathogenesis we are looking at **virulence factors that promote bacterial colonization of the host**. The following are virulence factors that promote bacterial colonization of the host .

1. The ability to use motility and other means to contact host cells and disseminate within a host.
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells.
4. The ability to compete for iron and other nutrients.
5. The ability to resist innate immune defenses such as phagocytosis and complement.
6. The ability to evade adaptive immune defenses.

We will now look at virulence factors that enable bacteria to adhere to host cells.

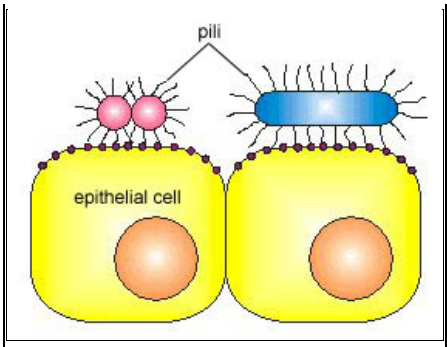
2. The Ability to Adhere to Host Cells and Resist Physical Removal

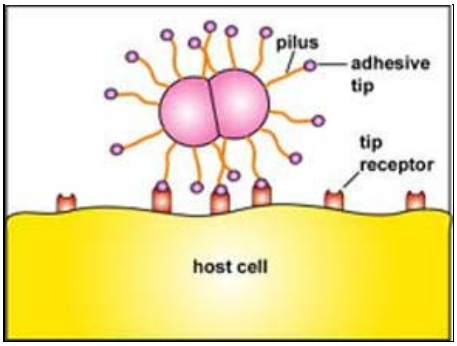
As we will see in Unit 5, one of the body's innate immune defenses is the ability to physically remove bacteria from the body through such means as the constant shedding of surface epithelial cells from the skin and mucous membranes, the removal of bacteria by such means as coughing, sneezing, vomiting, and diarrhea, and bacterial removal by bodily fluids such as saliva, blood, mucous, and urine. **Bacteria may resist this physical removal by producing pili, cell wall adhesin proteins, and/or biofilm-producing capsules**. In addition, the physical attachment of bacteria to host cells can also serve as a signal for the activation of genes involved in bacterial virulence. This process is known as signal transduction.

a. Using Pili (fimbriae) to Adhere to Host Cells

As seen in Unit 1, pili enable some organisms to **adhere** to receptors on target host cells **(see Fig. 1)** and thus **colonize and resist flushing** by the body. Pili are thin, protein tubes originating from the cytoplasmic membrane and are found in virtually all Gram-negative bacteria but not in many Gram-positive bacteria. The pilus has a shaft composed of a protein called pilin. At the end of the shaft is the **adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell (see Fig. 2)**. Because both the bacteria and the host cells have a negative charge, pili may enable the bacteria to bind to host cells without initially having to get close enough to be pushed away by electrostatic repulsion. Once attached to the host cell, the pili can depolymerize and enable adhesions in the bacterial cell wall to make more intimate contact.Th ere is also evidence that the binding of pili to host cell receptors can serve as a trigger for activating the synthesis of some cell wall adhesins.

Fig. 1: Illustration of Bacterial Adherence with Pili	Fig. 2: Illustration of Adhesive Tip of Bacterial Pili Binding to Host Cell Receptors





Flash animation showing a bacterium using both pili and cell wall adhesins to adhere to a host cell.

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

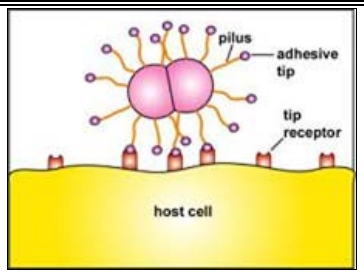
Pili enable some organisms to adhere to receptors on target host cells. The pilus has a shaft composed of a protein called pilin. At the end of the shaft is the adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell. Because both the bacteria and the host cells have a negative charge, pili may enable the bacteria to bind to host cells without initially having to get close enough to be pushed away by electrostatic repulsion. Once attached to the host cell, the pili can depolymerize and this enables bacterial cell wall adhesins to bind to adhesin receptors on the host cell. This allows the bacterial cell wall to make more intimate contact with the host cell and enables the bacterium to colonize the host cell and resist flushing. There is also evidence that the binding of pili to host cell receptors can serve as a trigger for activating the synthesis of some cell wall adhesins.

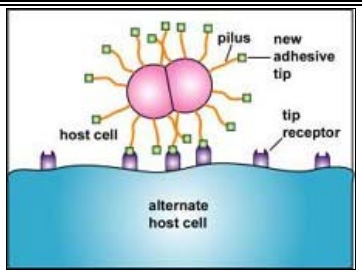
Animation of pathogenic *Escherichia coli* adhering to intestinal epithelial cells.

Courtesy of HHMI's Biointeractive.

Bacteria are constantly losing and reforming pili as they grow in the body and the **same bacterium may switch the adhesive tips of the pili** in order to adhere to different types of cells and evade immune defenses **(see Fig. 3)**. *E. coli*, for example, is able to make over 30 different types of pili.

Fig. 3: Illustration of Bacteria Altering the Adhesive Tips of Their Pili



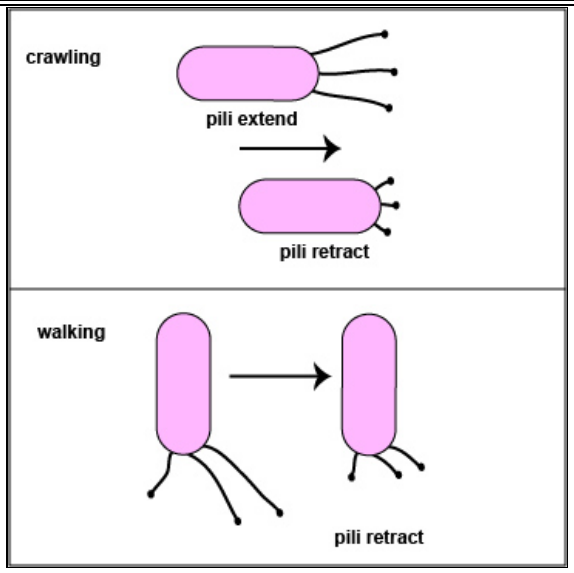


By genetically altering the adhesive tips of their pili, certain bacteria are able to: 1) adhere to and colonize different cell types with different receptors, and 2) evade antibodies made against the previous pili.

One class of pili, known as type IV pili, not only allows for attachment but also enable a twitching motility. They are located at the poles of bacilli and allow for a gliding motility along a solid surface such as a host cell. Extension and retraction of these pili allows the bacterium to drag itself along the solid surface **(see Fig. 4)**. In addition, bacteria can use their type IV pili to "slingshot" the bacterium over a cellular surface. In this case, as the pili contract they are thought to become

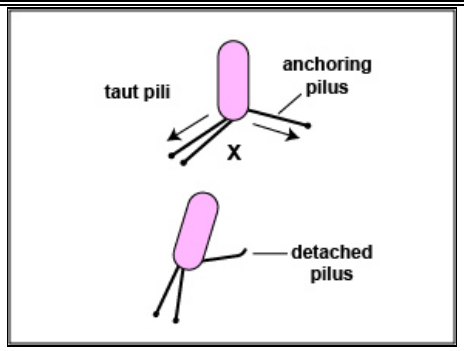
taut like a stretched rubber band. When an anchoring pilus detaches, the taut pili "slingshot" the bacterium in the opposite direction **(see Fig. 5)**. This motion typically alternates with the twitching motility and enables a more rapid motion and direction change than with the twitching motility because the rapid slingshotting motion reduces the viscosity of the surrounding biofilm. This enables bacteria with these types of pili within a biofilm to move around a cellular surface and find an optimum area on that cell for attachment and growth once they have initially bound. Bacteria with type IV pili include *Pseudomonas aeruginosa, Neisseria gonorrhoeae, Neisseria meningitidis*, and *Vibrio cholerae*.

Fig. 4: Illustration of Type IV Pili-Induced Dragging of a Bacterium



Some bacteria use type IV pili to move along surfaces within a biofilm. (Biofilms are to viscous for bacteria to swim through with flagella.) The bacterium extends and anchors its pili. As the pili retract the bacterium is pulled forward. This enables the bacterium to drag themselves through biofilms on environmental surfaces and alternates with a type-IV pili-induced slingshotting motion (see Fig. 5). The top illustration shows a bacterium dragging itself or "crawling" along a surface. Bacteria with polar pili are also able to pull themselves upright and "walk" along the surface as shown in the bottom illustration.

Fig. 5: Illustration of Type IV Pili-Induced Slingshotting of a Bacterium



Some bacteria use type IV pili to move along surfaces within a biofilm. (Biofilms are too viscous for bacteria to swim through with flagella.) A bacterium sitting at location "X" extends and anchors its pili. As the pili contract, the pili become stretched or taut. As the anchoring pilus on the right detaches, the taut pili "slingshot" the bacterium to the left. This motion alternates with the twitching motility (see Fig. 4) also caused by type IV pili and enables a more rapid motion and direction change than with the twitching motility because the rapid slingshotting motion reduces the viscosity of the surrounding biofilm.

Electron micrograph of type IV pili of *Neisseria gonorrhoeae* from Magdalene So, University of Arizona

Flash animation showing a bacterium using type IV pili to drag itself (twitching motility) along a surface.

html5 version of animation for iPad showing a bacterium using type IV pili to drag itself (twitching motility) along a surface.

Some bacteria use type IV pili to move along surfaces within a biofilm. (Biofilms are to viscous for bacteria to swim through with flagella.) The bacterium extends and anchors its pili. As the pili retract the bacterium is pulled forward. This enables the bacteria to drag themselves through biofilms on environmental surfaces with a twitching motility. This twitching motility alternates with a type-IV pili-induced slingshotting motion.

Flash animation showing a bacterium using type IV pili to slingshot itself along a surface.

html5 version of animation for iPad showing a bacterium using type IV pili to slingshot itself along a surface

Some bacteria use type IV pili to move along surfaces within a biofilm. (Biofilms are to viscous for bacteria to swim through with flagella.) It starts with a bacterium extending and anchoring its pili. As the pili contract, the pili become stretched or taut. As the anchoring pilus detaches, the taut pili "slingshot" the bacterium in the opposite direction. This motion alternates with the twitching motility also caused by type IV pili and enables a more rapid motion and direction change than with the twitching motility because the rapid slingshotting motion reduces the viscosity of the surrounding biofilm

Movie showing *Pseudomonas* using type IV pili to "walk" on end following binary fission.

Courtesy of Gerard Wong, UCLA Bioengineering, CNSI

Retraction of pili of *Pseudomonas* used in twitching motility.

Courtesy of Dr. Howard Berg, Roland Institute, Harvard University

Movie of twitching motility of *Pseudomonas* due to type IV pili.

Courtesy of Dr. Howard Berg, Roland Institute, Harvard University

For more information: Review of pili

Examples of bacteria using pili to colonize:

1. To cause infection, *Neisseria gonorrhoeae* must first colonize a mucosal surface composed of columnar epithelial cells. Pili allow for this initial binding and, in fact, *N. gonorrhoeae* is able to rapidly lose pili and synthesize new ones with a different adhesive tip, enabling the bacterium to adhere to a variety of tissues and cells including sperm, the epithelial cells of the mucous membranes lining the throat, genitourinary tract, rectum, and the conjunctiva of the eye. Subsequently, the bacterium is able to make more intimate contact with the host cell surface by way of a cell wall adhesin called Opa (see below).

Electron micrograph of type IV pili of *Neisseria gonorrhoeae* from Magdalene So, University of Arizona

2. The pili of *Neisseria meningitidis* allow it to adhere to mucosal epithelial cells in the nasopharynx where it is often asymptomatic. From there, however, it sometimes enters the blood and meninges and causes septicemia and meningitis. Type IV pili are thought to help the bacterium cross the blood brain barrier.

Highlighted Bacterium: *Neisseria meningitidis*

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

3. Uropathogenic strains of *Escherichia coli* can produce pili that enable the bacterium to adhere to the urinary epithelium and cause urinary tract infections. They also produce afimbrial adhesins (see below) for attachment to epithelial cells. Enteropathogenic *E.coli* (EPEC) use pili to adhere to intestinal mucosal cells.

Electron micrograph *E. coli* with pili, see Dennis Kunkel's Microscopy at the University of Hawaii-Manoa.

Electron micrographs of enteropathogenic E. coli (EPEC) adhering to intestinal cells, see Donnenberg Lab Images at the University of Maryland Medical School.

4. Pili of *Vibrio cholerae* allow it to adhere to cells of the intestinal mucosa and resist the flushing action of diarrhea.

5. Pili of *Pseudomonas aeruginosa* allow it to initially colonize wounds or the lung.

Flash animation showing bacteria lacking pili being flushed out of the urethra.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing bacteria lacking pili being flushed out of the urethra.
Without the pili the bacteria are flushed from the body.

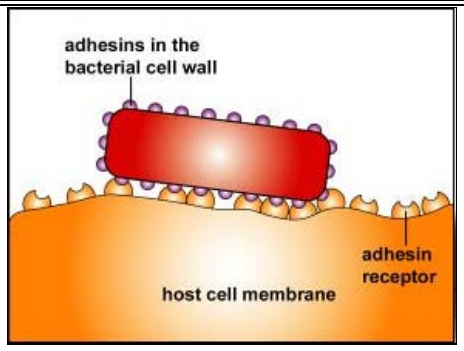
Flash animation showing how bacteria with pili resist being flushed out of the urethra.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing how bacteria with pili resist being flushed out of the urethra.
Bacterial pili bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make contact with the host cell, adhere, colonize, and resist flushing.

Concept map for Bacterial Colonization of Host Cell: Adhering to Host Cell to Resist Flushing.

b. Using Adhesins to Adhere to Host Cells

Adhesins are surface proteins found in the cell wall of various bacteria that bind to specific receptor molecules on the surface of host cells and enable the bacterium to **adhere intimately to that cell** in order to colonize and resist physical removal **(see Fig. 6**). Many, if not most bacteria probably use one or more adhesins to colonize host cells.

Fig. 6: Illustration of Bacterial Adhesins



Copyright © Gary E. Kaiser

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 cell wall adhesins to adhere to a host cell.

Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a bacterium using cell wall adhesins to adhere to a host cell.
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 resist being flushed out of the urethra.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a bacterium using adhesins to resist being flushed out of the urethra.
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 without adhesins being flushes out of the urethra.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing a bacterium without adhesins being flushes out of the urethra.
Without the necessary cell wall adhesins, the bacteria are flushed from the body.

For example:

1. *Streptococcus pyogenes* (group A beta streptococci) produce a number of adhesins:

   a. Protein F that binds to fibronectin, a common protein on epithelial cells. In this way it is able to adhere to the lymphatics and mucous membranes of the upper respiratory tract and cause streptococcal pharyngitis (strep throat).
   b. Lipoteichoic acid binds to fibronectin on epithelial cells.
   c. M-protein also functions as an adhesin.

2. The tip of the spirochete *Treponema pallidum* contains adhesins that are able to bind to fibronectin on epithelial cells.

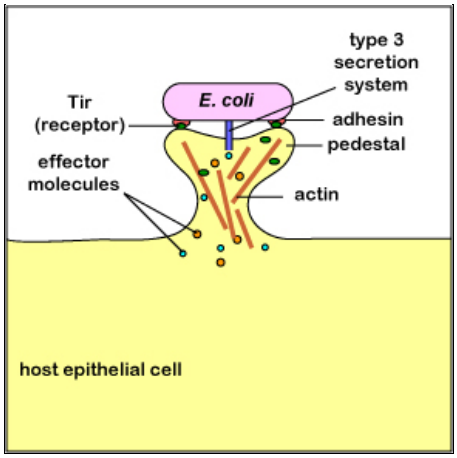
   Scanning electron Micrograph of *T. pallidum* adhering to a host cell by its tip.

3. The tip of the spirochete *Borrelia burgdorferi* contains adhesins that can bind to various host cells.

F**lash animation showing spirochetes using motility and invasins to enter a blood vessel.**
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing spirochetes using motility and invasins to enter a blood vessel.
A combination of motility and invasins appears to helps *Borrelia bergdorferi* and *Treponema pallidum* to invade and exit blood vessels by passing between and through endothelial cells. This enables these spirochetes to disseminate to other locations in the body. One tip of the spirochete attaches to the host cell and some form of invasin apparently causes the host cell to release digestive enzymes that enable the spirochete with its corkscrewing motility to penetrate the host cell membrane.

4. *Escherichia coli* O157 utilizes a type 3 secretion system to inject effector proteins into intestinal epithelial cells. Some of these cause polymerization of actin at the cell surface and this pushes the host cell cytoplasmic membrane up to form a pedestal. Another effector protein inserts into the membrane of the pedestal to serve as a receptor molecule for *E. coli* adhesins **(See Fig. 7)**.

Fig. 7: Illustration of *E. coli* Using a Type 3 Secretion System to Induce Pedestal Formation in a Host Cell



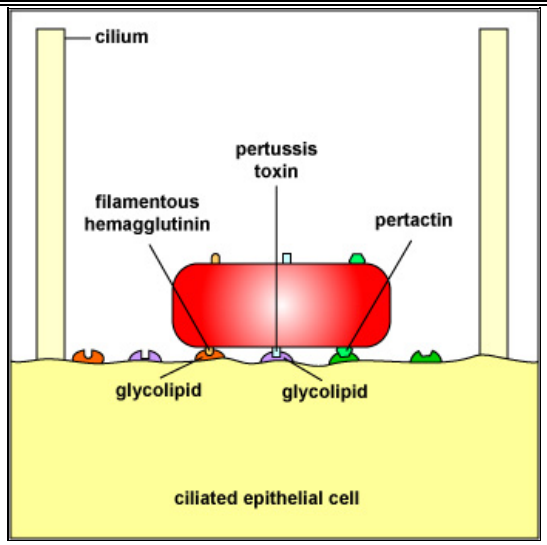
Escherichia coli O157 utilizes a type 3 secretion system to inject effector proteins into intestinal epithelial cells. Some of these cause polymerization of actin at the cell surface and this pushes the host cell cytoplasmic membrane up to form a pedestal. Another effector protein inserts into the membrane of the pedestal to serve as a receptor molecule for *E. coli* adhesins.

5. *Helicobacter pylori* use a type 4 secretion system to inject effector proteins into stomach epithelial cells to induce these host cells to display more receptors on their surface for *H. pylori* adhesins.

6. *Bordetella pertussis* produces several adhesins **(see Fig. 8)**:

a. Filamentous hemagglutinin is an adhesin that allows the bacterium to adhere to galactose residues of the glycolipids on the membrane of ciliated epithelial cells of the respiratory tract.
b. Pertussis toxin also functions as an adhesin. One subunit of the pertussis toxin remains bound to the bacterial cell wall while another subunit binds to the glycolipids on the membrane of ciliated epithelial cells of the respiratory tract.
c. *B. Pertussis* also produces an adhesin called pertactin that further enables the bacterium to adhere to cells.

Fig. 8: Illustration of *Bordetella pertussis* using Adhesins to Adhere to a Ciliated Epithelial Cell



Bordetella pertussis produces several adhesins:

Filamentous hemagglutinin is an adhesin that allows the bacterium to adhere to galactose residues of the glycolipids on the membrane of ciliated epithelial cells of the respiratory tract.

Pertussis toxin also functions as an adhesin. One subunit of the pertussis toxin remains bound to the bacterial cell wall while another subunit binds to the glycolipids on the membrane of ciliated epithelial cells of the respiratory tract.

B. Pertussis also produces an adhesin called pertactin that further enables the bacterium to adhere to cells.

7. *Neisseria gonorrhoeae* produces an adhesin called Opa (protein II) that enables the bacterium to make a more intimate contact with the host cell after it first adheres with its pili. Like with adhesive tips of pili, *N. gonorrhoeae* has multiple alleles for Opa protein adhesins enabling the bacterium to adhere to a variety of host cell types.

8. *Staphylococcus aureus* uses protein A as an adhesin to adhere to various host cells. It also helps the bacterium to resist phagocytosis.

Concept map for Bacterial Colonization of Host Cell: Adhering to Host Cell to Resist Flushing.

c. Using Biofilms to Adhere to Host Cells

Many normal flora bacteria produce a capsular polysaccharide matrix or glycocalyx to form a biofilm on host tissue. Biofilms are groups of bacteria attached to a surface and enclosed in a common secreted adhesive matrix, typically polysaccharide in nature. Many pathogenic bacteria, as well as normal flora and many environmental bacteria, form complex bacterial communities as biofilms.

Bacteria in biofilms are often able to communicate with one another by a process called quorum sensing 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.

For more information: Review of Quorum Sensing

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

Scanning electron micrograph of *Staphylococcus aureus* forming a biofilm in an indwelling catheter courtesy of CDC.

Biofilm of *Staphylococcus aureus* from Montana State University

For example:

1. *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 dextransucrase 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 initiate plaque formation. This dextran mesh traps the *S. mutans* and *S. sobrinus*, along with other bacteria and debris, and forms plaque. *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.

2. Most children suffering from chronic ear infection (otitis media) have a biofilm of bacteria in their middle ear. This biofilm contains bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* and enables the bacteria to chronically colonize the middle ear as well as resist body defenses and antibiotics. 3. Planktonic *Pseudomonas aeruginosa* 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 Slideshow Figs. 9A-9H)**.

Slideshow Activity

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.

Scanning electron micrograph of *Listeria* growing on a stainless steel surface. © Amy Lee Wong, author. Licensed for use, ASM MicrobeLibrary.

Scanning electron micrograph of *Pseudomonas* growing on bronchial mucosa. © Hiroyuki Kobayashi, author. Licensed for use, ASM MicrobeLibrary.

Scanning electron micrograph of *Staphylococcus aureus* forming a biofilm in an indwelling catheter courtesy of CDC.

Article and computer-generated model of biofilm formation courtesy of NIH.

For more information: Capsules and biofilms

TPS Questions

Concept map for Bacterial Colonization of Host Cell: Adhering to Host Cell to Resist Flushing.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Vibrio cholerae*
- *Streptococcus pyogenes*
- *Bordetella pertussis*
- *Salmonella* species
- *Helicobacter pylori*
- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- otitis media

Self Quiz for the Ability to Adhere to Host Cells and Resist Physical Removal

Quiz Group

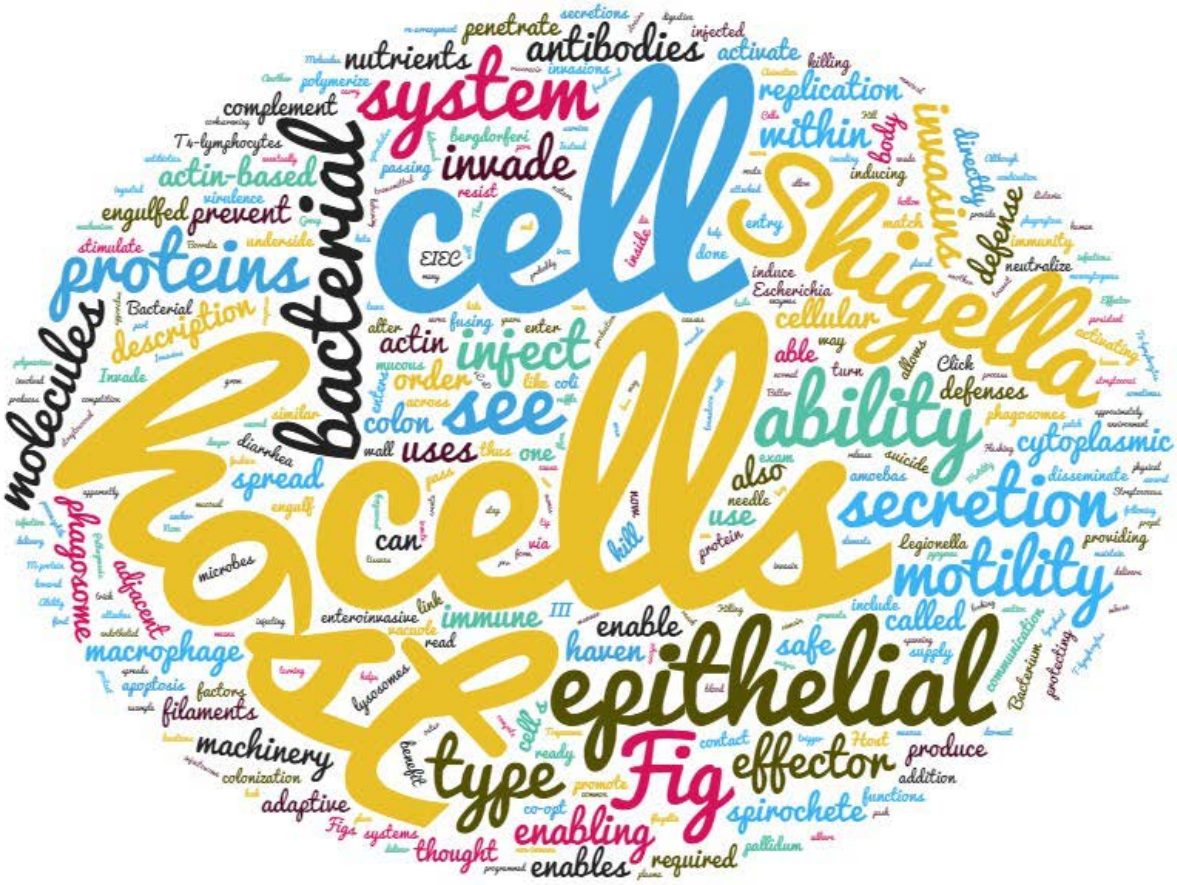
Ordering Activity

Back to Unit 3 Table of Contents

Back to Softchalk Lessons Table of Contents

The ability of some bacteria to invade host cells.
VIRULENCE FACTORS THAT PROMOTE BACTERIAL COLONIZATION OF THE HOST

3. The Ability to Invade Host Cells



Fundamental Statements for this Softchalk Lesson:

1. Some bacteria produce molecules called invasins that activate the host cell's cytoskeletal machinery enabling bacterial entry into the cell by phagocytosis.
2. Entering a non-defense host cell can provide the bacterium with a ready supply of nutrients, as well as protect the bacterium from complement, antibodies, and other body defense molecules.
3. Some bacteria invade phagocytic cells, neutralize their killing ability, and turn them into a safe haven for bacterial replication.
4. Some bacteria kill phagocytic dendritic cells once they are engulfed and prevent those dendritic cells from activating the T-lymphocytes required for adaptive immunity.
5. These bacteria 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.

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that promote microbial colonization of a host and give relevant examples.

Detailed Learning Objectives

1*. Briefly describe the mechanism by which invasins enable certain bacteria to enter host cells and state how this can promote colonization

2. Briefly describe how a type 3 secretion system might be used to invade and survive inside host cells.

3. State how certain pathogenic spirochetes such as *Treponema pallidum* and *Borrelia bergdorferi* use adhesins, invasins and motility to penetrate host cells.

(*) = Common theme throughout the course

(**) = More depth and common theme

Highlighted Bacterium

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

2. Read the description of *Salmonella* and match the bacterium with the description of the organism and the infection it causes.

3. Read the description of *Borrelia bergdorferi* and match the bacterium with the description of the organism and the infection it causes.

TPS Questions

In this Unit on Bacterial Pathogenesis we are looking at **virulence factors that promote bacterial colonization of the host**. The following are virulence factors that promote bacterial colonization of the host .

1. The ability to use motility and other means to contact host cells and disseminate within a host.
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells.
4. The ability to compete for iron and other nutrients.
5. The ability to resist innate immune defenses such as phagocytosis and complement.
6. The ability to evade adaptive immune defenses.

We will now look at virulence factors that enable bacteria to invade host cells.

3. The Ability to Invade Host Cells

Some bacteria produce molecules called **invasins** that **activate the host cell's cytoskeletal machinery enabling bacterial entry into the cell by phagocytosis**. Advantages of entering a human cell include:

a. **providing the bacterium with a ready supply of nutrient**s.

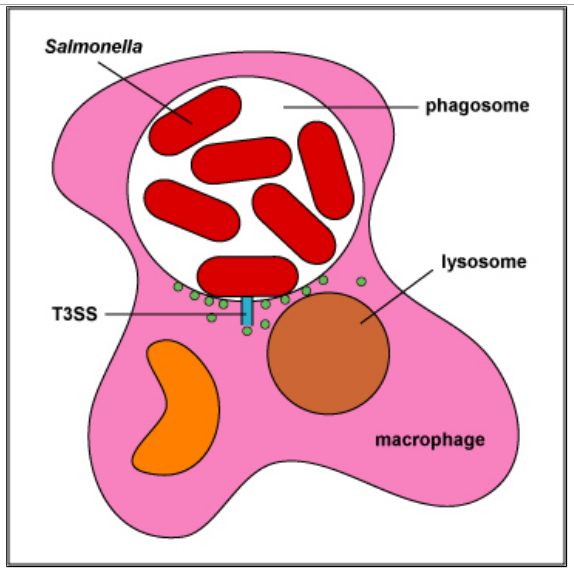
b. **protecting the bacteria from complement, antibodies, and other body defense molecules**.

In addition, some pathogenic bacteria:

a. **invade phagocytic cells, neutralize their killing ability, and turn them into a safe haven for bacterial replication (see Fig. 1)**.

b. **kill phagocytic** dendritic cells once they are engulfed and prevent those dendritic cells from activating the T4-lymphocytes and T8-lymphocytes required for adaptive immunity.

Fig. 1: Illustration of *Salmonella* Surviving Inside Macrophages

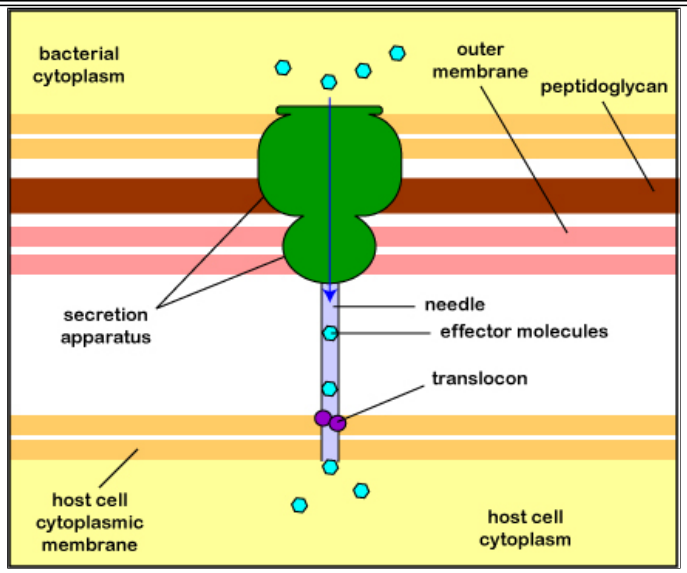


Once in the phagosome of the macrophage the bacterium uses its type 3 secretion system to inject proteins that prevent the lysosomes from fusing with the phagosomes, thus providing a safe haven for *Salmonella* replication within the phagosome and protecting the bacteria from antibodies and other defense elements.

Invasins of *Salmonella, Shigella*, and enteroinvasive strains of *Escherichia coli* (EIEC), for example, allow these bacteria to enter epithelial cells of the colon. These bacteria, like many 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 **(see Fig. 2)**. 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 injectisome.

Fig. 2: Illustration of The Bacterial Type 3 Secretion System



Many bacteria involved in infection have the ability to co-opt the functions of the host cell to the benefit of the bacterium. 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 injectisome.

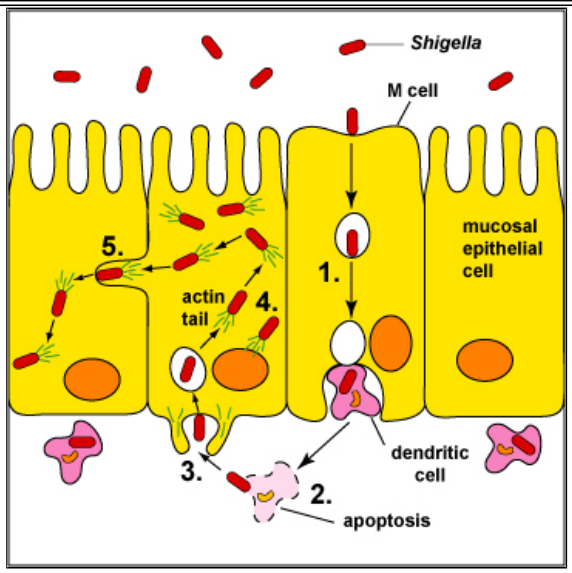
When these bacteria contact the epithelial cells of the colon, the type III secretion system delivers proteins into the epithelial cells **enabling them to polymerize and depolymerize actin filaments**. This cytoskeletal rearrangement is a key part of the pseudopod formation in phagocytic cells and is what enables phagocytes to engulf bacteria and place them in a vacuole. Thus the bacterium with its invasins is able to trick the epithelial cell into behaving like a phagocyte and engulfing the bacterium. The bacteria then replicate within the host cell.

Flash animation of a bacterium using a type 3 secretion system to secrete invasins in order to penetrate non-immune host cells.
Copyright © Gary E. Kaiser
html5 version of animation for iPad of a bacterium using a type 3 secretion system to secrete invasins in order to penetrate non-immune host cells.
Some bacteria use a type 3 secretion system system to secrete invasins into a host cell in order to activate the host cell's cytoskeletal machinery and enabling bacterial entry into the cell by phagocytosis. By entering the cytoplasm of the host cell, it has a ready supply of nutrients and is able to protect the bacteria from complement, antibodies, and certain other body defenses.

We will now look at several examples of bacteria that use invasions to invade host cells.

1. It is thought that *Shigella* first transit the mucous membrane of the colon by passing through M cells. (M cells are phagocytic cells in the mucous membrane whose function is to sample microbes from the intestinal lumen and pass them on to the lymphoid tissue of the Peyer's patch in order to activate the immune defenses against intestinal microbes). Once across the mucosa, the *Shigella* use a **type 3 secretion system** to inject invasins into the underside of the epithelial cells to induce phagocytic uptake of the bacterium **(see Fig. 3)**.

Fig. 3: Illustration of *Shigella* Passing Through the Mucous Membrane and Invading Mucosal Epithelial Cells Via M-Cells



Copyright © Gary E. Kaiser

A proposed model for invasion of epithelial cells of the colon.

1) The Shigella first cross the mucosa by passing through specialized cells called M cells. The M cell passes the Shigella on to a dendritic cell.

2) The Shigella subsequently escapes from the dendritic cell by inducing apoptosis, a programmed cell suicide.

3) The Shigella then uses its invasins to enter the mucosal epithelial cells from underneath. The invasins cause actin polymer rearrangements in the cytoskeleton of the host cell resulting in the bacterium being engulfed and placed in an endocytic vesicle in a manner similar to phagocytic cells. Once inside, the Shigella escape from the vacuole into the cytoplasm and multiply.

4) The Shigella are able to move through the host cell and spread to adjacent host cells by a unique process called actin-based motility. In this process, actin filaments polymerize at one end of the bacterium, producing comet-like tails that propel the Shigella through the cytoplasm of the host cell.

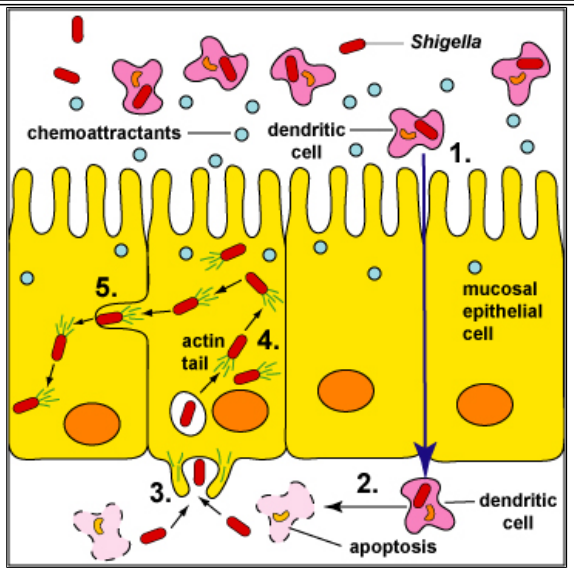
5) When they reach the boundary of that cell, the actin filaments push the Shigella across that membrane and into the adjacent cell.

Once inside they escape from the vacuole into the cytoplasm and multiply. Once inside, *Shigella* produces a protease that cleaves tubulin, a major component of the microtubule cytoskeleton. The microtubules represent a barrier to bacterial movement within the infected cell and the protease breaks down this barrier.

Now they move through the host cell and spread to adjacent host cells by a unique process called **actin-based motility** whereby actin filaments polymerize at one end of the bacterium producing comet-like tails that propel the *Shigella* through the cytoplasm of the host cell. When they reach the boundary of that cell, the actin filaments push the *Shigella* across that membrane and into the adjacent cell **(see Fig. 3)**. Actin-based motility enables the bacteria to spread from cell-to-cell without having to encounter defense cells and antibodies. As the *Shigella* grow and spread within the epithelial cells, those epithelial cells die and provoke a strong inflammatory response leading to the symptoms of dysentery.

In addition, *Shigella* can induce the host cells to produce signaling molecules that attract phagocytic, antigen-presenting dendritic cells to the area. It enters the dendritic cells and uses them to carry the *Shigella* through the intestinal wall to the underside. It then uses its type 3 secretion system to inject effector proteins from the phagosome into the cytoplasm. These proteins trigger apoptosis or cell suicide of the dendritic cell. Killing the dendritic cells prevents them from presenting *Shigella* to T4-lymphocytes, a step required for the production of antibodies against the *Shigella* **(see Fig. 4)**.

Fig. 4: Illustration of *Shigella* Passing Through the Mucous Membrane and Invading Mucosal Epithelial Cells Via Dendritic Cells



A proposed model for invasion of epithelial cells of the colon.

1) The Shigella first cross the mucosa by passing through specialized cells called M cells. The M cell passes the Shigella on to a dendritic cell.

2) The Shigella subsequently escapes from the dendritic cell by inducing apoptosis, a programmed cell suicide.

3) The Shigella then uses its invasins to enter the mucosal epithelial cells from underneath. The invasins cause actin polymer rearrangements in the cytoskeleton of the host cell resulting in the bacterium being engulfed and placed in an endocytic vesicle in a manner similar to phagocytic cells. Once inside, the Shigella escape from the vacuole into the cytoplasm and multiply.

4) The Shigella are able to move through the host cell and spread to adjacent host cells by a unique process called actin-based motility. In this process, actin filaments polymerize at one end of the bacterium, producing comet-like tails that propel the Shigella through the cytoplasm of the host cell.

5) When they reach the boundary of that cell, the actin filaments push the Shigella across that membrane and into the adjacent cell.

A movie showing *Shigella* being propelled by actin-based motility within a cell, courtesy of the Theriot Lab Website at Stanford University Medical School.

The phase-luscent streaks behind the bacteria are the actin-rich comet tails.

Speeded up 300X.

GIF animation of Shigella invading an intestinal mucosal epithelial cell

Highlighted Bacterium: *Shigella*

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

2. *Salmonella* use a type 3 secretion system to inject intestinal epithelial cells with effector proteins that stimulate actin re-arrangement and cause the epithelial cell's cytoplasmic to "ruffle" up and engulf the bacteria (see Slideshow Figs. 5A - Fig. 5B). The *Salmonella* pass through the epithelial cell where they are engulfed by phagocytic macrophages.

Slideshow Activity

Once in the phagosome of the macrophage the bacterium uses its type 3 secretion system to inject proteins that prevent the lysosomes from fusing with the phagosomes, thus providing a safe haven for *Salmonella* replication within the phagosome and protecting the bacteria from antibodies and other defense elements **(see Fig. 5C-5D)**. By injecting flagellin into the cytoplasm of the macrophage the *Salmonella* can also eventually kill the macrophage by inducing apoptosis, a programmed cell suicide.

Slideshow Activity

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.

Molecules injected into the intestinal epithelial cells also stimulate diarrhea. Advantages of inducing diarrhea include:

a. **Flushing out normal flora bacteria so there is less competition for nutrients**; and

b. **Better enabling *Salmonella* that are not attached to host cells to be transmitted to a new host via the fecal-oral rout**e.

A movie showing *Salmonella* invading a human cell, courtesy of the Theriot Lab Website at Stanford University Medical School.
Actin-rich ruffles on the host cell cytoplasmic membrane engulf the bacteria. Speeded up 450X.

Highlighted Bacterium: *Salmonella*
Click on this link, read the description of *Salmonella*, and be able to match the bacterium with its description on an exam.

3. *Listeria monocytogenes* is another bacterium that enters intestinal cells via invasins and spreads to adjacent cells by actin-based motility. Its actin-based motility enables it to moves approximately 1.5 µm per second within the host cell.

A movie showing *Listeria* entering host cells and being propelled by actin-based motility within a cell, courtesy of the Theriot Lab Website at Stanford University Medical School.
The phase-dense streaks behind the bacteria are the actin-rich comet tails. Speeded up 150X.

4. Although enteroinvasive *Escherichia coli* (EIEC) don't have actin-based motility, they invade and kill epithelial cells of the colon in a manner similar to *Shigella*.

5. *Legionella pneumophila*, after being ingested by macrophages and placed in a phagosome, uses a type 4 secretion system to inject effector proteins that prevent the lysosomes from fusing with the phagosomes and turning the macrophage into a safe haven for bacterial replication. The same mechanism allows the *Legionella* to survive inside amoebas in nature. These amoebas serve as a reservoir for the bacterium in the environment.

6. F protein and M-protein of *Streptococcus pyogenes* (Group A beta streptococci) enables the bacterium to invade epithelial cells. This is thought to help maintain persistent streptococcal infections and enable the bacterium to spread to deeper tissues.

7. The spirochete *Borrelia bergdorferi* probably uses a combination of **invasins and motility** to penetrate host cells. In this case the host cell doesn't phagocytose the bacterium. Instead, one tip of the spirochete attaches to the host cell and some form of invasin apparently causes the host cell to release digestive enzymes that enable the spirochete with its corkscrewing motility to penetrate the host cell membrane. Once in the host cell the bacteria may remain dormant for years and hide from the immune system and antibiotics.

8. Another spirochete, *Treponema pallidum*, is thought to enter cells in a similar fashion. Motility also helps *B. bergdorferi* and *T. pallidum* to invade and leave blood vessels by passing between and through endothelial cells, thus enabling the spirochetes to disseminate to other locations in the body.

Electron micrograph of *Treponema pallidum* invading a host cell.

Flash animation showing spirochetes using motility and invasins to enter a blood vessel.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing spirochetes using motility and invasins to enter a blood vessel.
A combination of motility and invasins appears to helps *Borrelia bergdorferi* and *Treponema pallidum* to invade and exit blood vessels by passing between and through endothelial cells. This enables these spirochetes to disseminate to other locations in the body. One tip of the spirochete attaches to the host cell and some form of invasin apparently causes the host cell to release digestive enzymes that enable the spirochete with its corkscrewing motility to penetrate the host cell membrane.

Highlighted Bacterium: *Borrelia bergdorferi*

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

TPS Questions

Concept map for Bacterial Colonization of Host Cell: Invading the Host Cell

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Shigella* species
- *Listeria monocytogenes*
- *Escherichia coli*
- *Salmonella* species
- *Pseudomonas aeruginosa*
- *Legionella pneumophilia*
- *Yersinia enterocolitica*
- *Neisseria gonorrhoeae*
- *Borrelia burgdorferi*
- *Treponema pallidum*
- *Streptococcus pneumoniae*

Self Quiz for the Ability to Invade Host Cells

The ability of some bacteria to invade host cells.

Quiz Group

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The ability of bacteria to compete for iron and other nutrients

4. The Ability to Compete for Iron and Other Nutrients

Fundamental Statements for this Softchalk Lesson:

1. The ability to be pathogenic is directly related to the bacterium's ability to compete successfully with host tissue and normal flora for limited nutrients.
2. Bacteria compete for nutrients by synthesizing specific transport systems or cell wall components capable of binding limiting substrates and transporting them into the cell.
3. Iron is an essential nutrient for both bacterial growth and human cell growth. Both bacteria and their host synthesize compounds capable of binding iron for their use.
4. Certain bacteria have developed means of "stealing" iron from other bacteria and/or from host cells.

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that promote microbial colonization of a host and give relevant examples.

Detailed Learning Objectives

1.* State why the ability to compete for iron and other nutrients is important for bacteria to cause disease and describe briefly three ways bacteria may accomplish this as part of their pathogenicity.

(*) = Common theme throughout the course

In this Unit on Bacterial Pathogenesis we are looking at **virulence factors that promote bacterial colonization of the host**. The following are virulence factors that promote bacterial colonization of the host .

1. The ability to use motility and other means to contact host cells and disseminate within a host.
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells.
4. The ability to compete for iron and other nutrients.
5. The ability to resist innate immune defenses such as phagocytosis and complement.
6. The ability to evade adaptive immune defenses.

We will now look at virulence factors that enable bacteria to compete for iron and other nutrients.

4. The Ability to Compete for Iron and Other Nutrients

Often the ability to be pathogenic is directly related to the bacterium's ability to compete successfully with host tissue and normal flora for limited nutrients. One reason the generation time of bacteria growing in the body is substantially slower than in lab culture is because essential nutrients are limited. In fact this is a major reason why the overwhelming majority of bacteria found in nature are not harmful to humans.

To be pathogenic, a bacterium must be able to multiply in host tissue.The more rapid the rate of replication, the more likely infection may be established. Pathogens, therefore, are able to compete successfully for limited nutrients in the body. Generally **bacteria compete for nutrients by synthesizing specific transport systems or cell wall components capable of binding limiting substrates and transporting them into the cell**. A good example of this is the ability of bacteria to compete for iron.

For more information: The bacterial cytoplasmic membrane

As we will see later in Unit 5 under innate immunity, the body makes considerable metabolic adjustment during infection to deprive microorganisms of iron. **Iron is essential for both bacterial growth and human cell growth**. **Bacteria synthesize iron chelators - compounds capable of binding iron** - called siderophores. Many siderophores are excreted by the bacterium into the environment, bind free iron, and then re-enter the cell and release the iron. Other siderophores are found on the cell wall where they bind iron and transport it into the bacterium.

Meanwhile, **the body produces iron chelators of its own** (transferrin, lactoferrin, ferritin, and hemin) so the concentration of free iron is very low. **The ability of bacterial iron chelators to compete successfully with the body's iron chelators as well as those of normal flora may be essential to pathogenic bacteria**.

In addition to their own siderophores, some bacteria:

1. Produce **receptors for siderophores of other bacteria** in this way take iron from other bacteria;

2. 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;

3. **Produce proteases that degrade human lactoferrin, transferrin, or heme to release the bound iron** for capture by bacterial siderophores;

4. **Don't use iron as a cofactor**. *Borrelia burgdorferi* instead uses manganese as a cofactor;

5. Are able to **produce exotoxins that kill host cells only when iron concentrations are low**. In this way the bacteria can **gain access to the iron** that was in those cells.

Staphylococcus aureus, on the other hand, produces surface adhesins that bind to extracellular matrix proteins and polysaccharides surrounding host cell tissue, including fibronectin, collagen, laminin, hyaluronic acid, and elastin. ***S. aureus* proteases and hyaluronidase then dissolve these components of the extracellular matrix providing food for the bacteria and enabling the bacteria to spread**.

Concept map for Bacterial Colonization of Host Cell: Compete for Nutrients such as iron.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Neisseria meningitidis*
- *Neisseria gonorrhoeae*
- *Haemophilus influenzae*
- *Yersinia* species

- *Borrelia burgdorferi*

Self Quiz for the Ability to Compete for Iron and Other Nutrients

Quiz Group

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An overview of phagocytosis, the complement pathways, and antibacterial peptides
VIRULENCE FACTORS THAT PROMOTE BACTERIAL COLONIZATION OF THE HOST

5. The Ability to Resist Innate Immune Defenses such as Phagocytosis, the Complement Pathways, and Antibacterial Peptides

a. An Overview of Phagocytosis, the Complement Pathways, and Antibacterial Peptides

Fundamental Statements for this Softchalk Lesson:

1. For phagocytosis to occur, the surface of the microbe must be attached to the cytoplasmic membrane of the phagocyte through unenhanced or enhanced attachment.
2. Following attachment, the microbe must be engulfed and placed on a membrane-bound vesicle called a phagosome. The phagosome then becomes acidified to provide the correct pH for killing by lysosomal enzymes.
3. Lysosomes, containing digestive enzymes and microbicidal chemicals, fuse with the phagosome containing the ingested microbe and the microbe is destroyed. This is referred to as intracellular killing by phagocytes and happens when microbial numbers are relatively low.
4. If the the infection site contains very large numbers of microorganisms and high levels of inflammatory cytokines and chemokines are being produced, the phagocyte will empty the contents of its lysosomes by a process called degranulation in order to kill the microorganisms extracellularly. This is referred to as extracellular killing.
5. The body's complement pathways consist of a variety of complement proteins that when activated participate in four important body defense functions: promoting inflammation, phagocyte chemotaxis, opsonization (enhanced attachment), and lysis of membrane-bound cells.
6. The body produces a number of antibacterial peptides that are directly toxic by forming pores in the cytoplasmic membrane of a variety of microorganisms causing leakage of cellular needs.

Common Course Objectives

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

2. Describe the stages of phagocytosis

Detailed Learning Objectives

1**. Describe the following as they relate to phagocytosis:

a. unenhanced attachment
b. enhanced attachment
c. ingestion
d. destruction

2*. State 4 different body defense functions of the body's complement pathways.

3*. State what is meant by antibacterial peptides and give an example.

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Questions

In this Unit on Bacterial Pathogenesis we are looking at **virulence factors that promote bacterial colonization of the host**. The following are virulence factors that promote bacterial colonization of the host .

1. The ability to use motility and other means to contact host cells and disseminate within a host.
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells.
4. The ability to compete for iron and other nutrients.
5. The ability to resist innate immune defenses such as phagocytosis and complement.
6. The ability to evade adaptive immune defenses.

We will now look at virulence factors that enable bacteria to resist innate immune defenses such as phagocytosis, the complement pathways, and antibacterial peptides. **We will begin with an overview of these innate immune defenses**.

5. The Ability to Resist Innate Immune Defenses such as Phagocytosis and Complement

a. An Overview of Phagocytosis, the Complement Pathways, and Antibacterial Peptides

1. An Overview of Phagocytosis

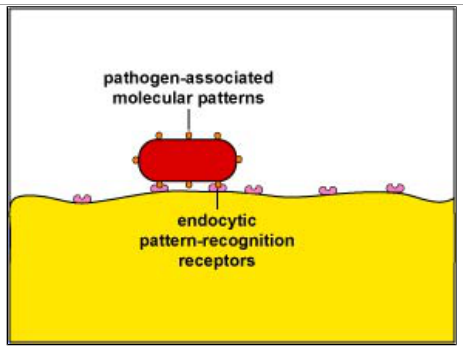
As will be seen in Unit 5, there are several steps involved in phagocytosis.

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.

1. 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 Fig. 1)**.

Fig. 1: Illustration of 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.

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

html5 version of animation for iPad illustrating the function of endocytic pattern-recognition receptors on phagocytes.

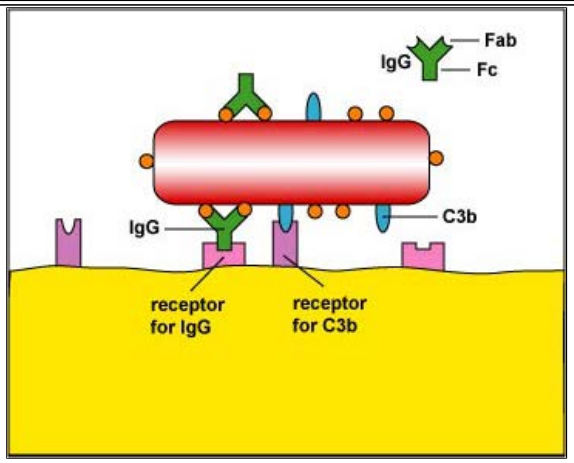
Glycoprotein molecules known as endocytic pattern-recognition receptors are found on the surface of phagocytes. They are so named because they recognize and bind to pathogen-associated molecular patterns - molecular components associated with microorganisms but not found as a part of eukaryotic cells. 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 pathogen-associated molecular patterns (PAMPs)

For more information: Preview of pattern-recognition receptors

2. **Enhanced attachment** is the attachment of microbes to phagocytes by way of molecules such as an antibody molecule called IgG and two proteins produced during the complement pathways called C3b and C4b **(see Fig. 2)**. Molecules such as IgG, C3b, and C4b that promote enhanced attachment are called **opsonins** and the process is called **opsonization**. Enhanced attachment is much more specific and efficient than unenhanced.

Fig. 2: Illustration of 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.

Flash animation illustrating the function of enhanced attachment by way of IgG.
Copyright © Gary E. Kaiser
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.

For more information: Preview of antibodies

For more information: Preview of the benefits of the complement pathways

b. **Ingestion**

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 (see Slideshow Fig. 3A and Fig. 3B)**.

Slideshow Activity

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 hydrogen ions (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**.

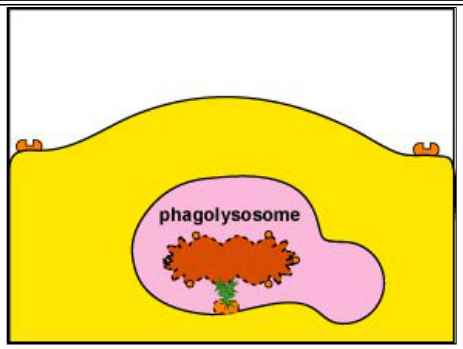
Flash animation showing acidification of the phagosome following ingestion.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing acidification of the phagosome following ingestion.
During phagosome formation, an electron pump brings protons (H^+) into the phagosome. This lowers the 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.

c. **Destruction**

1. **Intracellular destruction**

Finally, lysosomes, **containing digestive enzymes and microbicidal chemicals, fuse with the phagosome** containing the ingested microbe and the microbe is destroyed **(see Fig. 5)**.

Fig. 5: Illustration of Fusion of Phagosome and Lysosome



The lysosome its digestive enzymes and microbicidal chemicals fuses with the phagosome containing the ingested bacteria to form a phagolysosome and the bacterium is killed.

Flash animation showing intracellular destruction by a phagocyte.

html5 version of animation for iPad showing intracellular destruction by a phagocyte.

Lysosomes move along the cytoskeleton and fuse with phagosomes to form phagolysosomes.

2. **Extracellular destruction**

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**.

Flash animation summarizing phagocytosis through unenhanced attachment.

html5 version of animation for iPad summarizing phagocytosis through 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 through enhanced attachment.

html5 version of animation for iPad summarizing phagocytosis through enhanced attachment.

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.

To view a **scanning electron micrograph of a macrophage with pseudopods and phagocytosis of *E. coli* by a macrophage** on a blood vessel, see Dennis Kunkel's Microscopy, University of Hawaii-Manoa.

Concept map for Bacterial Colonization of Host Cell: An Overview of Phagocytosis and the Complement Pathways

2. An Overview of the Body's Complement Pathways

Some bacteria are able to **interfere with the body's complement pathways**. The complement pathways will be discussed in detail later in Unit 4, but a brief summary is relevant here.

There are three complement pathways: the classical complement pathway, the alternative complement pathway, and the lectin pathway. While the three pathways differ in the way they are activated, once activated they all produce the same beneficial complement proteins. Basically the **complement proteins are a series of serum proteins that when activated participate in four important body defense functions**. These include:

a. Inflammation

Inflammation is **the means by which body defense cells and defense chemicals leave the blood and enter the tissue around an injured or infected site**. Complement proteins known as C5a, C3a, and C4a lead to **vasodilation**, increased capillary permeability, and the expression of the adhesion molecules on leukocytes and the vascular endothelium. This **enables leukocytes to adhere to the inner wall of the capillaries, pass between the endothelial cells, and enter the surrounding tissue**. Vasodilation also **enables a variety of defense chemicals in the plasma of the blood to enter the tissue.Th ese** defense chemicals include antibodies and complement proteins. C5a also causes neutrophils to release proteases and toxic oxygen radicals for extracellular killing of microbes.

Flash animation of a capillary prior to vasodilation.

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

Flash animation of a capillary showing vasodilation.
Copyright © Gary E. Kaiser
html5 version for iPad of animation of a capillary showing vasodilation.
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.

b. Phagocyte Chemotaxis

Complement proteins C3a and C5a are chemoattractants for leukocytes. **Chemotaxis enables the phagocytes to move toward the infected area in order to remove microorganisms**.

Flash animation summarizing early inflammation and diapedesis.
Copyright © Gary E. Kaiser
html5 version for iPad of animation 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 up regulation 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 up regulates 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 are attracted towards chemotactic agents such as the complement protein C5a and leukotriene B_4 generated by cells at the site of infection or injury.

Flash animation summarizing late inflammation and diapedesis.
Copyright © Gary E. Kaiser
html5 version for iPad of animation 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 an maintains the inflammatory response by up regulation 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.

Movie showing chemotaxis by neutrophils.

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

Movie showing chemotaxis by neutrophil as they remove dead liver cells.
From Science Friday on NPR; originally published in Science by Paul Kubes and colleagues.

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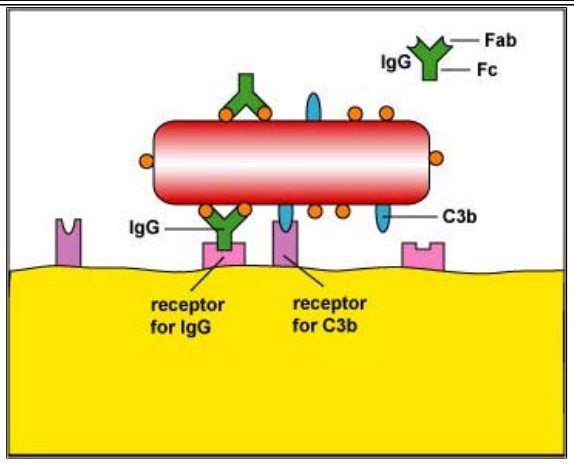
c. Opsonization (Enhanced Attachment)

The complement proteins **C3b and C4b are known as opsonins because they bind microbes to phagocytes (see Fig. 2)**. One portion of the molecule binds to microbial proteins while the other portion binds to receptors on phagocytes. In this way, microbes can be engulfed by phagocytes more effectively.

Fig. 2: Illustration of 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.

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.

html5 version for iPad of 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.

During the complement pathways, complement proteins such as C3a, C3b, C4a, C4b, and C5a are produces. 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).

d. MAC Lysis of Biological Membranes

A series of complement proteins known as the membrane attack complex or MAC put pores in cellular membranes resulting in lysis. This is used to kill such things as Gram-negative bacteria, virus-infected cells, and tumor cells.

For more information: Preview of the Complement pathways

Flash animation showing the formation of the membrane attack complex (MAC) during the complement pathways.

html5 version for iPad of animation showing the formation of the membrane attack complex (MAC) during the complement pathways.

The membrane attack complex (MAC), produced by the complement pathways, 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.

Concept map for Bacterial Colonization of Host Cell: An Overview of Phagocytosis and the Complement Pathways

TPS Questions

3. Antibacterial Peptides

The body produces a number of antibacterial peptides such as human defensins and cathelicidins that are directly toxic by forming pores in the cytoplasmic membrane of a variety of microorganisms causing leakage of cellular needs. 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.

For more information: Preview of antibacterial peptides and enzymes

Some bacteria are able to **resist phagocytosis,interfere with the body's complement pathways, and resist antibacterial peptides**. In the next two sections we will look at the following virulence factors:

1. **The ability to resist phagocytic engulfment (attachment and ingestion)**
2. **The ability to resist phagocytic destruction and serum lysis**

Self Quiz for an Overview of Phagocytosis, the Complement Pathways, and Antibacterial Peptides

Quiz Group

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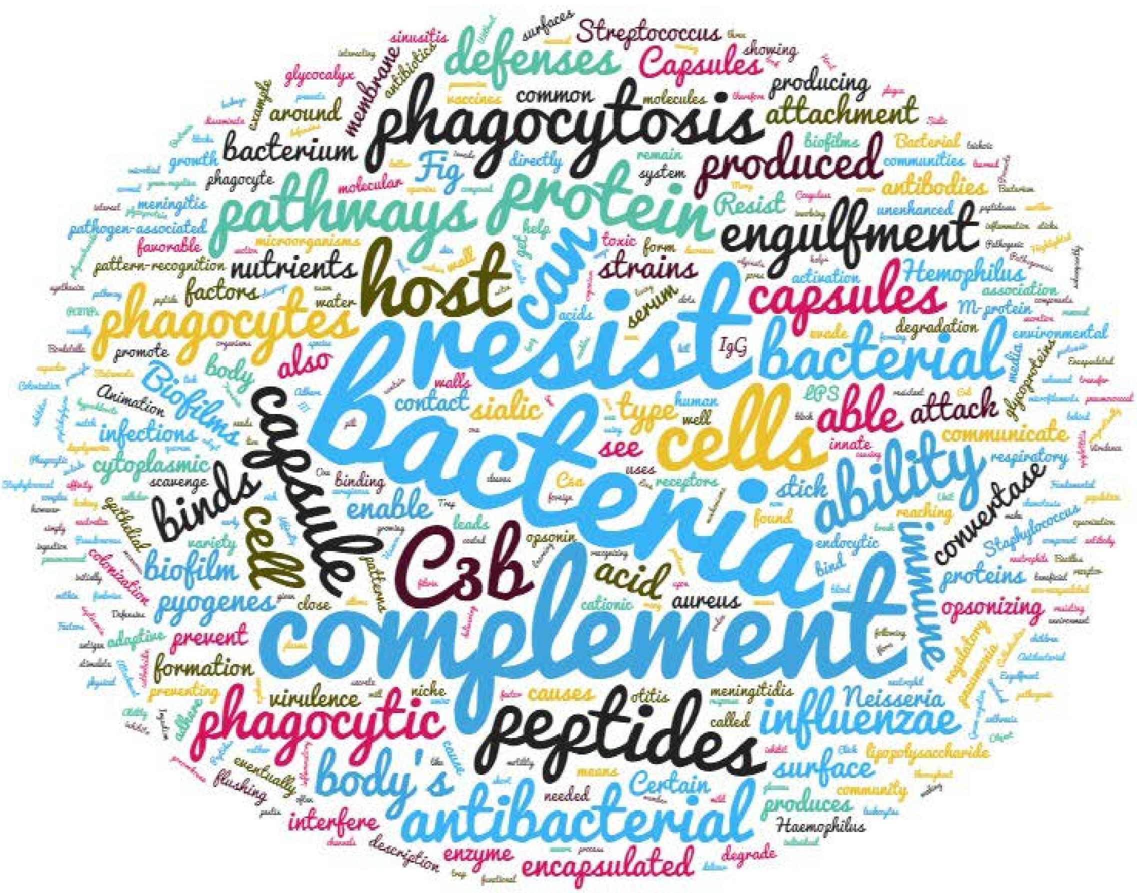
The ability of bacteria to resist phagocytic engulfment and antibacterial peptides
VIRULENCE FACTORS THAT PROMOTE BACTERIAL COLONIZATION OF THE HOST

5. The Ability to Resist Innate Immune Defenses such as Phagocytosis, Complement, and Antibacterial peptides

b. The Ability to Resist Phagocytic Engulfment (Attachment and Ingestion) and Antibacterial Peptides



Fundamental Statements for this Softchalk Lesson:

1. ***Capsules can resist unenhanced attachment by by preventing pathogen-associated molecular patterns or from binding to endocytic pattern-recognition receptors on the surface of the phagocytes.***
2. ***The capsules of some bacteria interfere with the body's complement pathway defenses.***
3. ***The body's immune defenses can eventually get around the capsule by producing opsonizing antibodies (IgG) against the capsule that stick the capsule to the phagocyte. This is the principle behind some vaccines.***
4. ***Biofilms enable bacteria 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.***
5. ***Certain bacteria can resist antibacterial peptides.***

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that promote microbial colonization of a host and give relevant examples.

Detailed Learning Objectives

1*. Briefly **describe** at least 3 ways capsules may enable bacteria to resist phagocytic engulfment and state how this can promote colonization.

2. State at least 2 mechanisms other than capsules that certain bacteria might use to resist phagocytic engulfment.

3. State 3 ways bacteria might resist antibacterial peptides like defensins.

(*) = Common theme throughout the course

(**) = More depth and common theme

Highlighted Bacterium

1. Read the description of *Haemophilus influenzae* and match the bacterium with the description of the organism and the infection it causes.

TPS Questions

In this Unit on Bacterial Pathogenesis we are looking at **virulence factors that promote bacterial colonization of the host**. The following are virulence factors that promote bacterial colonization of the host .

1. The ability to use motility and other means to contact host cells and disseminate within a host.
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells.
4. The ability to compete for iron and other nutrients.
5. The ability to resist innate immune defenses such as phagocytosis and complement.
6. The ability to evade adaptive immune defenses.

We will now look at virulence factors that enable bacteria to resist phagocytic engulfment and antibacterial peptides.

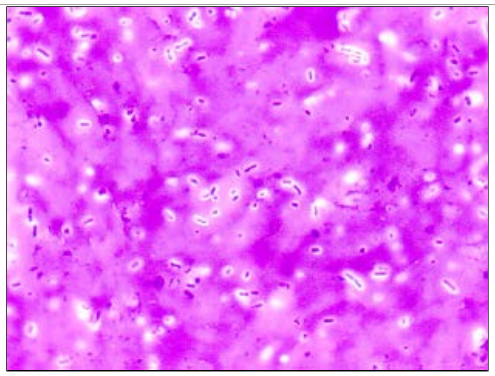
b. The Ability to Resist Phagocytic Engulfment (Attachment and Ingestion) and Antibacterial Peptides

For more information: Preview of phagocytosis

For more information: Preview of the complement pathways

As we learned in Unit 1, **capsule**s enable many organisms to **resist phagocytic engulfment**. For example, *Streptococcus pneumoniae* is able to initially evade phagocytosis and cause infections such as pneumococcal pneumonia, sinusitis, otitis media, and meningitis because of its capsule. Encapsulated strains of *Haemophilus influenzae* type b can causes severe respiratory infections, septicemia, epiglottitis, and meningitis in children. (Other non-encapsulated strains of *H. influenzae* usually cause mild respiratory infections such as sinusitis and otitis media.) Other encapsulated bacteria include *Neisseria meningitidis*, *Bacillus anthracis*, and *Bordetella pertussis*.

Photomicrograph of an Encapsulated Bacterium



Note the clear capsules surrounding the bacilli.

Highlighted Bacterium: *Haemophilus influenzae*

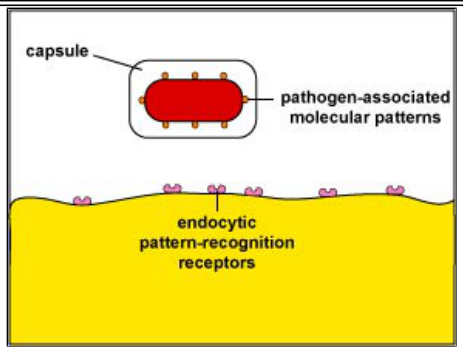
Click on this link, read the description of *Haemophilus influenzae*, and be able to match the bacterium with its description on an exam.

For more information: Review of capsules and biofilms

Movie of a bacterium being engulfed by a neutrophil.	**Movie of a bacterium resisting being engulfed by a neutrophil.**
Phagocytosis. © James Sullivan, author. Licensed for use, ASM MicrobeLibrary.	Phagocytosis. © James Sullivan, author. Licensed for use, ASM MicrobeLibrary.

1. **Capsules can resist unenhanced attachment** by preventing **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 - from binding to **endocytic pattern-recognition receptors** on the surface of the phagocytes **(see Fig. 1)**.

Fig. 1: Illustration of Capsules Blocking the Unenhanced Attachment of Bacteria to 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 - components of common molecules such as peptidoglycan, teichoic acids, lipopolysaccharide, mannans, and glucans - found in many microorganisms. Capsules can cover up these surface molecules preventing their attachment to the endocytic pattern-recognition sites on the phagocyte.

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

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

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.

2. **The capsules of some bacteria interfere with the body's complement pathways**. Capsules can interfere with the complement pathways in a number of ways:

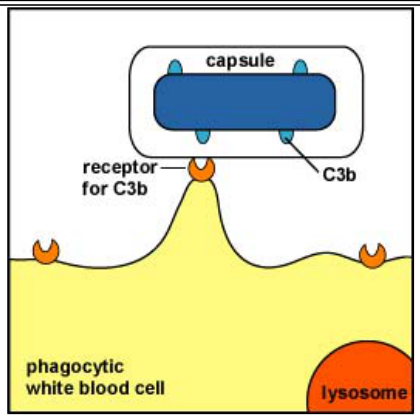
a. **Some capsules prevent the formation of C3 convertase**, an early enzyme in the complement pathways. Without this enzyme, **the opsonins C3b and C4b involved in enhanced attachment, as well as the other beneficial complement proteins like C5a, are not produced**.

b. **Some capsules are rich in sialic acid**, a common component of host cell glycoprotein. Sialic acid **has an affinity for serum protein H**, a complement regulatory protein that leads to the degradation of the opsonin C3b and the formation of C3 convertase. (Our body uses serum protein H to degrade any C3b that binds to host cell glycoproteins so that we don't stick our own phagocytes to our own cells with C3b.) Some *Neisseria meningitidis* strains synthesize their own sialic acid capsule. While *Neisseria gonorrhoeae* and *Hemophilus influenzae* type b do not have a sialic acid capsule, they are able to scavenge sialic acid from host cells and enzymatically transfer it to their surface where it subsequently binds protein H.

c. **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. 2**). This is seen with the capsule of *Streptococcus pneumoniae*.

d. *Staphylococcus aureus* produces a protein called Staphylococcal complement inhibitor that binds and inhibits the C3 convertase enzyme needed for all three complement pathways.

Fig. 2: Illustration of Bacterial Capsule Preventing C3b Receptors on Phagocytes from Binding to C3b Attached to a Bacterial Cell Wall



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

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

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.

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 pneumococccal 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.

Flash animation illustrating phagocytosis of an encapsulated bacterium through opsonization.

html5 version of animation for iPad illustrating phagocytosis of an encapsulated bacterium through opsonization.

The Fab portion of IgG binds to epitopes of a capsule. The Fc portion can now attach the capsule to Fc receptors

on phagocytes for enhanced attachment. Once attached to the phagocyte by way of IgG, the encapsulated bacterium can be engulfed more efficiently and placed in a phagosome.

3. **Biofilms**

Many pathogenic bacteria, as well as normal flora, form complex bacterial communities as biofilms. Bacteria in biofilms are often able to communicate with one another by a process called quorum sensing 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.

For example, *Pseudomonas aeruginosa* produces a glycocalyx composed of alginate. This enables strains producing the glycocalyx to block neutrophil chemotaxis, scavenge the hypochlorite molecules produced by neutrophils to kill bacteria, decrease phagocytosis, and inhibit activation of the complement pathways.

Concept map for Bacterial Colonization of Host Cell: Resisting Phagocytosis, Complement, and Antibacterial peptides

4. **Certain bacteria can resist phagocytic engulfment and complement using mechanisms not involving capsules**.

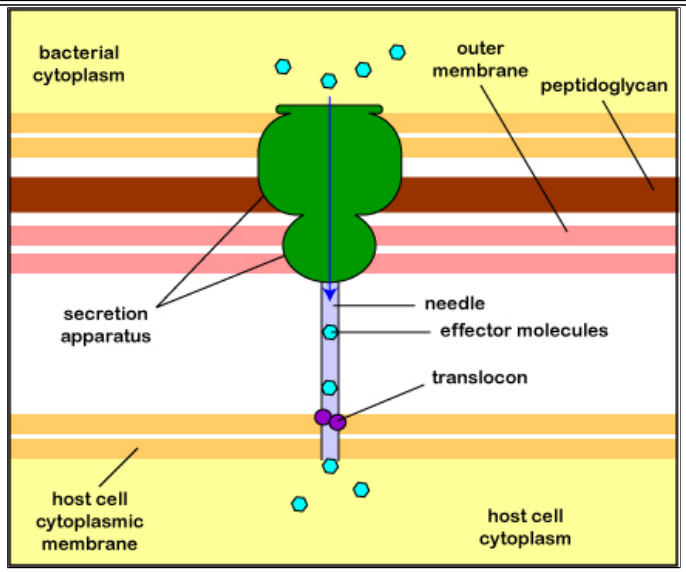
a. The M-protein of *Streptococcus pyogenes* allows these bacteria to be more resistant to phagocytic engulfment. The **M-protein** of *S. pyogenes* **binds factor H,** a complement regulatory protein that leads to the degradation of the opsonin C3b and the formation of C3 convertase. (Our body uses serum protein H to degrade any C3b that binds to host cell glycoproteins so that we don't stick our own phagocytes to our own cells with C3b.) *S. pyogenes* also produces a protease that **cleaves the complement protein C5a**.

b. **Coagulase**, produced by *Staphylococcus aureus*. Coagulase causes **fibrin clots** to form around the organism that help **enable it to resist phagocytosis**. Our adaptive immune system has difficulty in recognizing the *S. aureus* as foreign when it is coated with a human protein.

c. Pathogenic *Yersinia*, such as the species that causes plague, *Y. pestis*, contact phagocytes and, by means of a type III secretion system **(see Fig. 3)**, **deliver proteins that depolymerize the actin microfilaments needed for phagocytic engulfment into the phagocytes (see Fig.4)**.

d. The pili (fimbriae) of *Streptococcus pyogenes* both blocks the activation of the complement pathways on the bacterial cell wall and helps to resist phagocytic engulfment.

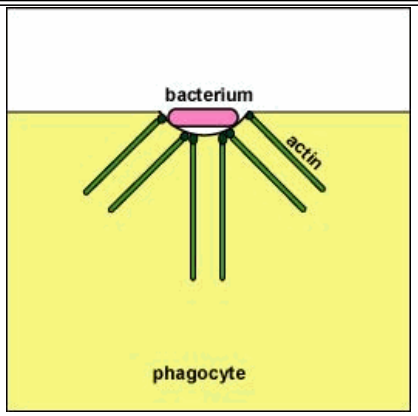
Fig. 3: Illustration of the Bacterial Type 3 Secretion System



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.

Fig. 4: Illustration of depolymerization of actin



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

GIF animation showing bacteria depolymerizing actin in order to resist phagocytosis

Concept map for Bacterial Colonization of Host Cell: Resisting Phagocytosis, Complement, and Antibacterial peptides

TPS Questions

5. Certain bacteria can resist antibacterial peptides

Human defensins are short cationic peptides 29-34 amino acids long that are directly toxic by forming pores in the cytoplasmic membrane of a variety of microorganisms causing leakage of cellular needs. 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.

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 pepitide also can bind to and neutralize LPS from gram-negative cell walls to reduce inflammation.

a. **Capsules help prevent antibacterial peptides from reaching the cytoplasmic membrane of some bacteria**.

b. T**he lipopolysaccharide (LPS) of the Gram-negative cell wall binds cationic antibacterial peptides and prevents them from reaching the cytoplasmic membrane**.

c. **Some bacteria secrete peptidases that break down antibacterial peptides**.

Concept map for Bacterial Colonization of Host Cell: Resisting Phagocytosis, Complement, and Antibacterial peptides

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Streptococcus pneumoniae*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Bacillus anthracis*
- *Neisseria gonorrhoeae*
- *Streptococcus pyogenes*
- *Staphylococcus aureus*
- *Yersinia pestis*
- *Pseudomonas aeruginosa*

Self Check for the Ability to Resist Phagocytic Engulfment (Attachment and Ingestion) and Antibacterial Peptides

Quiz Group

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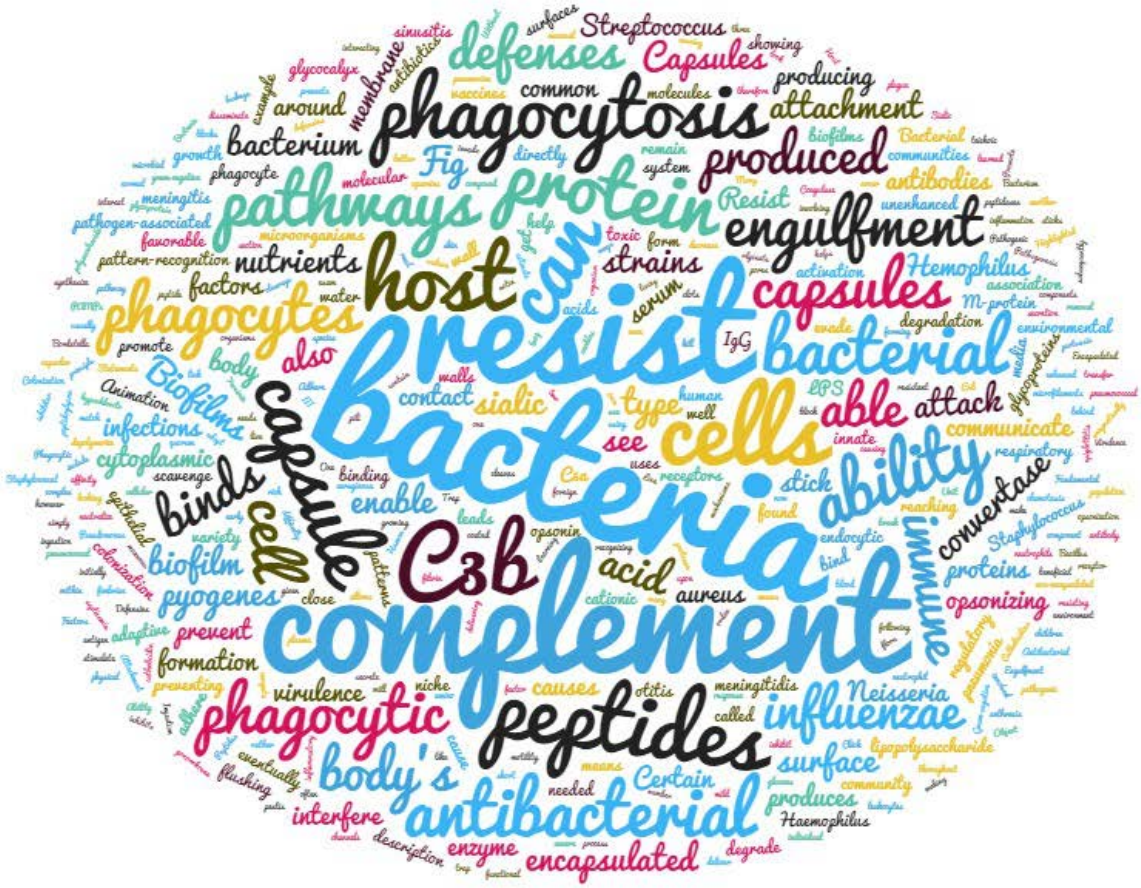
The ability of bacteria to resist phagocytic destruction
VIRULENCE FACTORS THAT PROMOTE BACTERIAL COLONIZATION OF THE HOST

5. The Ability to Resist Innate Immune Defenses such as Phagocytosis, Complement, and Antibacterial peptides

c. The Ability to Resist Phagocytic Destruction



Fundamental Statements for this Softchalk Lesson:

1. ***Some bacteria resist phagocytic destruction by preventing fusion of the lysosome with the phagosome.***
2. ***Some bacteria resist phagocytic destruction by escaping from the phagosome before the lysosome fuses.***
3. ***Some bacteria resist phagocytic destruction by preventing acidification of the phagosome.***
4. ***Some bacteria resist phagocytic destruction by resisting killing by lysosomal chemicals.***
5. ***Some bacteria resist phagocytic destruction by killing phagocytes.***

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that promote microbial colonization of a host and give relevant examples.

Detailed Learning Objectives

1*. State at least 4 different ways bacteria might be able to resist phagocytic destruction once engulfed.

(*) = Common theme throughout the course

TPS Questions

In this Unit on Bacterial Pathogenesis we are looking at **virulence factors that promote bacterial colonization of the host**. The following are virulence factors that promote bacterial colonization of the host .

1. The ability to use motility and other means to contact host cells and disseminate within a host.
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells.
4. The ability to compete for iron and other nutrients.
5. The ability to resist innate immune defenses such as phagocytosis and complement.
6. The ability to evade adaptive immune defenses.

We will now look at virulence factors that enable bacteria to resist phagocytic destruction.

c. The Ability to Resist Phagocytic Destruction

For more information: Preview of phagocytosis

For more information: Preview of the complement pathways

Bacteria resist phagocytic destruction by a variety of means.

a. Resisting phagocytic destruction: preventing fusion of the lysosome with the phagosome.

Once *Salmonella* is engulfed by macrophages and placed in a phagosome, the bacterium **uses its type 3 secretion system to inject proteins that prevent the lysosomes from fusing with the phagosomes**, thus providing a safe haven for *Salmonella* replication within the phagosome and protecting the bacteria from antibodies and other defense elements **(see Fig. 1)**.

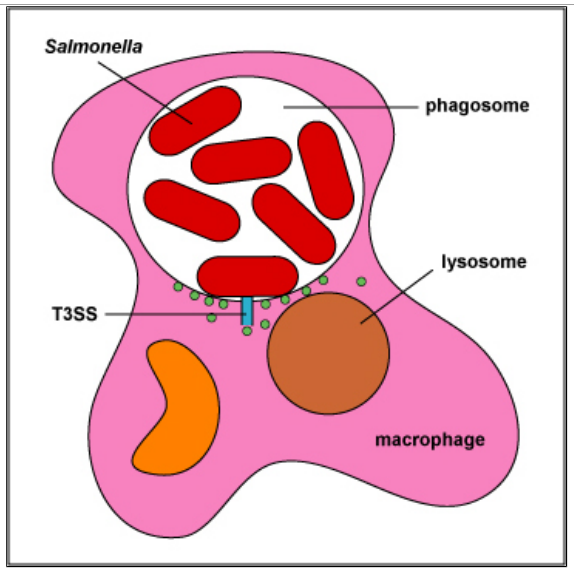
Legionella pneumophila, after being ingested by macrophages and placed in a phagosome, **uses a type 4 secretion system to inject effector proteins that prevent the lysosomes from fusing with the phagosomes** and turning the macrophage into a safe haven for bacterial replication.

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

Cell wall lipids of *Mycobacterium tuberculosis*, such as lipoarabinomannan, **arrest the maturation of phagosomes preventing delivery of the bacteria to lysosomes**.

Some bacteria, such as species of *Salmonella*, *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Chlamydia trachomatis,* **block the vesicular transport machinery that enables the lysosome to move to the phagosome for fusion.**

Fig. 1: *Salmonella* Surviving Inside Macrophages



Once in the phagosome of the macrophage the bacterium uses its type 3 secretion system to inject proteins that prevent the lysosomes from fusing with the phagosomes, thus providing a safe haven for *Salmonella* replication within the phagosome and protecting the bacteria from antibodies and other defense elements.

Flash animation showing a bacterium resisting phagocytosis by blocking fusion of the phagosome with the lysosomes.

html5 version of Flash animation for iPad showing a bacterium resisting phagocytosis by blocking fusion of the phagosome with the lysosomes.

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.

Flash animation showing a bacterium resisting phagocytosis by blocking the lysosomes from moving to the phagosome

html5 version of animation for iPad showing a bacterium resisting phagocytosis by blocking the lysosomes 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.

b. Resisting phagocytic destruction: escaping from the phagosome

Some bacteria, such as *Shigella flexneri*, *Listeria monocytogenes*, 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.

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.

c. Resisting phagocytic destruction: preventing acidification of the phagosome.

Some bacteria, such as *Mycobacterium tuberculosis* and *Legionella pneumophilia,* **prevent the acidification of the phagosome that 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 (acid hydrolases) 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.

d. Resisting phagocytic destruction: resisting killing by lysosomal chemicals

Some bacteria, such as *Salmonella*, are **more resistant to toxic forms of oxygen and to defensins** , the toxic peptides that kill bacteria by damaging their cytoplasmic membranes.

The **carotenoid pigments** that give *Staphylococcus aureus* species its golden color and group B streptococci (GBS) its orange tint **shield the bacteria from the toxic oxidants that neutrophils use to kill bacteria**.

e. Resisting phagocytic destruction: killing the phagocyte

Some bacteria are able to **kill phagocytes**. Bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* produce the exotoxin **leukocidin** that damages either the cytoplasmic membrane of the phagocyte or the membranes of the lysosomes, resulting in the phagocyte being killed by its own enzymes.

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 either the cytoplasmic membrane of the phagocyte or the membranes of the lysosomes, resulting in death of the phagocyte.

TPS Questions

Concept Map for Bacterial Colonization of Host Cell: Resisting Phagocytic Destruction

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Legionella pneumophilia*
- *Salmonella* species
- *Shigella* species
- *Rickettsia rickettsii*
- *Mycobacterium tuberculosis*
- *Legionella pneumophilia*
- *Chlamydia trachomatis*
- *Streptococcus pyogenes*
- *Staphylococcus aureus*
- *Neisseria*

Self Quiz for the Ability to Resist Phagocytic Destruction

Self Check

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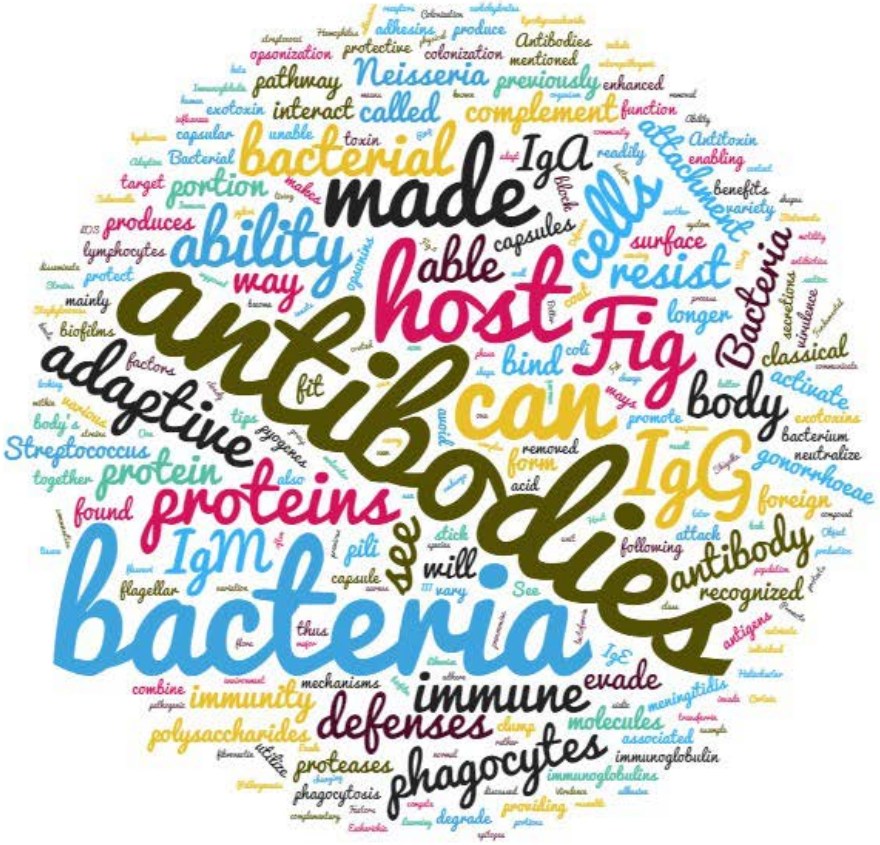
The ability of bacteria to evade adaptive immunity
VIRULENCE FACTORS THAT PROMOTE BACTERIAL COLONIZATION OF THE HOST

6. The Ability to Evade Adaptive Immune Defenses



Fundamental Statements for this Softchalk Lesson:

1. There are various ways that the antibodies the body makes during adaptive immunity protect the body against bacteria.
2. Some antibodies such as IgG and IgE function as opsonins and stick bacteria to phagocytes (opsonization or enhanced attachment).
3. Antibodies, such as IgG, IgA, and IgM, can bind to bacterial adhesins, pili, and capsules and in this way block their attachment to host cells.
4. IgG and IgM can activate the classical complement pathway providing all of its associated benefits.
5. IgA and IgM can clump bacteria together enabling them to be more readily removed by phagocytes.
6. Antitoxin antibodies, mainly IgG, are made against bacterial exotoxins. They combine with the exotoxin molecules before they can interact with host target cells and thus neutralize the toxin.
7. Bacteria utilize a variety of mechanisms to resist antibodies made during adaptive immunity.
8. Some bacteria can vary their surface proteins or polysaccharides so that antibodies previously made against those proteins will no longer "fit."
9. Some bacteria are able to coat themselves with host proteins and in this way avoid having antibodies being made against them because they are unable to be recognized as foreign
10. Some bacteria produce immunoglobulin proteases that degrade the body's protective antibodies (immunoglobulins) that are found in body secretions.

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that promote microbial colonization of a host and give relevant examples.

4. Describe the different ways in which antibodies play a role in removing and/or neutralizing microbes and toxins.

Detailed Learning Objectives

1*. State 4 four ways the antibody molecules made during adaptive immunity protect us against bacteria.

2*. Briefly describe at least 3 ways a bacterium might evade our adaptive immune defenses and name a bacterium that does each.

(*) = Common theme throughout the course

TPS Questions

In this Unit on Bacterial Pathogenesis we are looking at **virulence factors that promote bacterial colonization of the host**. The following are virulence factors that promote bacterial colonization of the host .

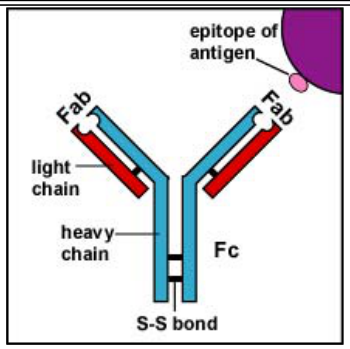
1. The ability to use motility and other means to contact host cells and disseminate within a host.
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells.
4. The ability to compete for iron and other nutrients.
5. The ability to resist innate immune defenses such as phagocytosis and complement.
6. The ability to evade adaptive immune defenses.

We will now look at virulence factors that enable bacteria to resist adaptive immunity.

6. The Ability to Evade Adaptive Immune Defenses

One of the major defenses against bacteria is the immune defenses' **production of antibody molecules against the organism**. The "tips" of the antibody, called the Fab portion **(see Fig. 1 and Fig. 5A)** have shapes that are complementary to portions of bacterial proteins and polysaccharides called epitopes. The "bottom" of the antibody, called the Fc portion **(see Fig. 1)** binds to receptors on phagocytes and NK cells and can activate the classical complement pathway.

Fig. 1: Normal Antibody-Antigen Reaction



The Fab portion of the antibody has specificity for binding an epitope of an antigen. The Fc portion directs the biological activity of the antibody. In the case 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.

There are various ways that the antibodies the body makes during adaptive immunity protect the body against bacteria:

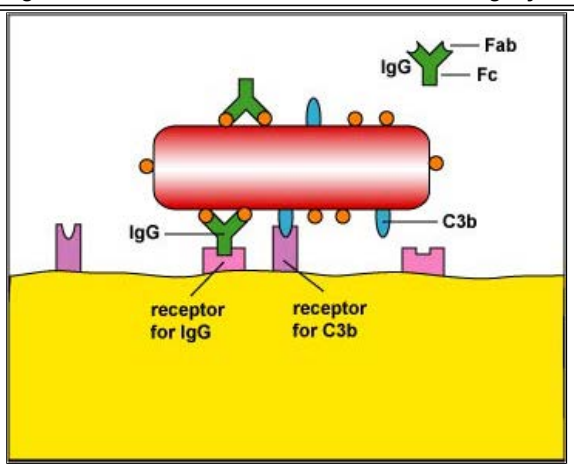
a. As mentioned above under phagocytosis, some antibodies such as IgG and IgE function as opsonins and **stick bacteria to phagocytes (see Fig. 2)**.

b. Antibodies, such as IgG, IgA, and IgM, can bind to bacterial adhesins, pili, and capsules and in this way **block their attachment to host cells**.

c. IgG and IgM can also **activate the classical complement pathway** providing all of its associated benefits.

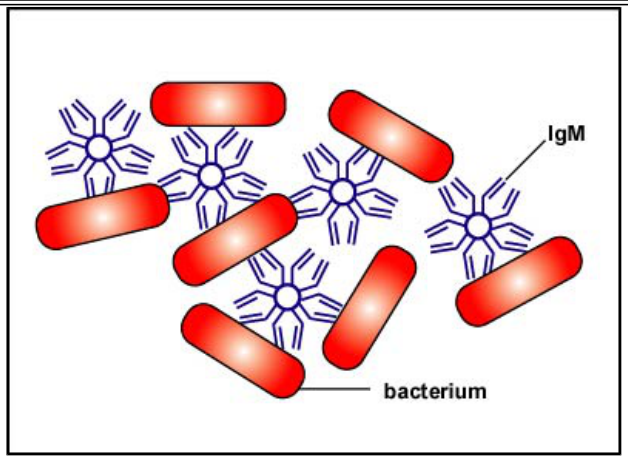
d. IgA and IgM can **clump bacteria together** enabling them to be more readily removed by phagocytes **(see Fig. 3)**.

Fig. 2: 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.

Fig. 3: Agglutination of Microorganisms



The multiple Fab portions of IgM link microorganism together so out of the lymph and blood and phagocytosed more effectively.

Flash animation illustrating enhanced attachment by way of IgG.

html5 version of animation for iPad illustrating 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 antibodies blocking bacterial adherence to host cell.

html5 version of animation for iPad showing antibodies blocking bacterial adherence to host cell.

The Fab portion of the antibodies made against epitopes of adherence structures such as cell wall adhesins bind and block the bacteria from adhering to receptors on the host cell membrane. As a result, the bacteria are unable to colonize and may be flushed away.

For more information: Preview of antibodies

TPS Questions

Bacteria utilize a variety of mechanisms to resist antibodies made during adaptive immunity. These include the following:

a. Certain bacteria can evade antibodies is by **changing the adhesive tips of their pili** as mentioned above with *Escherichia coli* and *Neisseria gonorrhoeae* **(see Fig. 4)**. Bacteria can also **vary other surface proteins** so that antibodies previously made against those proteins will no longer "fit." **See Fig. 5A and Fig. 5B**. For example, *N. gonorrhoeae* produces Rmp protein (protein III) that protects against antibody attack by antibodies made against other surface proteins (such as adhesins) and the lipooligosaccharide (LOS) of the bacterium.

b. Strains of *Neisseria meningitidis* have a **capsule composed of sialic acid** while strains of *Streptococcus pyogenes* (group A beta streptococci) have a **capsule made of hyaluronic acid**. Both of these polysaccharides closely resemble carbohydrates found in human tissue and because they are not recognized as foreign by the lymphocytes that carry out the adaptive immune responses, **antibodies are not made against those capsules**. Likewise, some bacteria are able to **coat themselves with host proteins** such as fibronectin, lactoferrin, or transferrin and in this way avoid having antibodies being made against them because they are unable to be recognized as foreign by lymphocytes.

c. *Staphylococcus aureus* produces protein A while *Streptococcus pyogenes* produces protein G. Both of these proteins bind to the Fc portion of the antibody IgG, the portion that is supposed to bind the bacterium to phagocytes during enhanced attachment **(see Fig. 1)**. The bacteria become coated with antibodies in a way that does not result in opsonization **(see Fig. 6)**.

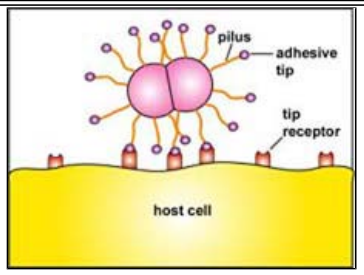
d. *Salmonella* species can undergo phase variation of their capsular (K) and flagellar (H) antigens, that is, they can change the molecular shape of their capsular and flagellar antigens so that antibodies made against the previous form no longer fit the new form. **See Fig. 5A and Fig. 5B**.

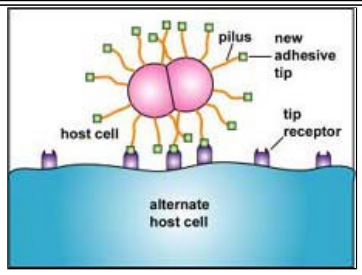
e. Bacteria such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Helicobacter pylori*, *Shigella flexneri*, *Neisseria meningitidis*, *Neisseria gonorrhoeae* and enteropathogenic *E. coli* produce **immunoglobulin proteases**. Immunoglobulin proteases **degrade the body's protective antibodies (immunoglobulins) that are found in body secretions**, a class of antibodies known as IgA.

f. Many pathogenic bacteria, as well as normal flora, form complex bacterial communities as biofilms. Bacteria in biofilms are often able to communicate with one another by a process called quorum sensing (discussed later in this unit) 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:

1. better able to resist attack by antibiotics, and
2. are better able to resist the host immune system.

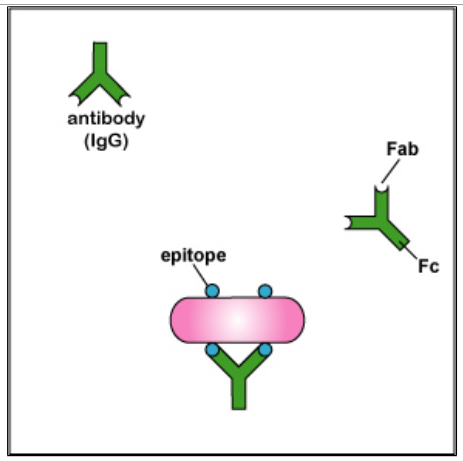
Fig. 4: Bacteria Altering the Adhesive Tips of Their Pili



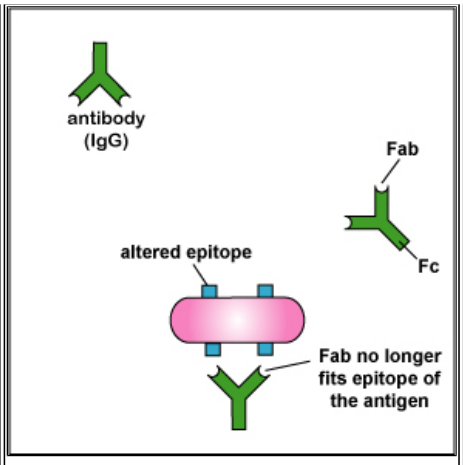


By genetically altering the adhesive tips of their pili, certain bacteria are able to: 1) adhere to and colonize different cell types with different receptors, and 2) evade antibodies made against the previous pili.

Fig. 5A: Normal Antibody-Antigen Reaction	Fig. 5B: Altering Epitopes of an Antigen in order to Resist Antibody Molecules

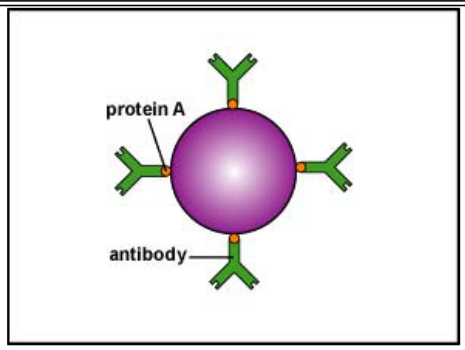


The Fab portion of the antibody has specificity for binding an epitope of an antigen. An epitope is the portion of an antigen - such as a few amino acids sticking out of a protein - to which the Fab portion of an antibody molecule fits. The Fc portion of an antibody directs the biological activity of the antibody. In the case of IgG, the Fc portion can bind to phagocytes for enhanced attachment (opsonization) as well as activate the classical complement pathway.



The Fab portion of the antibody has specificity for binding an epitope of an antigen. By altering the molecular shape of an epitope of an antigen through mutation or genetic recombination, previous antibody molecules against the original shaped epitope no longer fit or bind to the antigen.

Fig. 6: *Staphylococcus aureus* Resisting Opsonization via Protein A



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.

Concept Map for Bacterial Colonization of Host Cell: Resisting Adaptive Immunity

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Streptococcus pyogenes*
- *Staphylococcus aureus*
- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- *Helicobacter pylori*
- *Shigella* species
- *Escherichia coli*

Self Quiz for the Ability to Evade Adaptive Immune Defenses

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The ability of PAMPs to trigger the production of inflammatory cytokines that result in an excessive inflammatory response
VIRULENCE FACTORS THAT DAMAGE THE HOST

C. VIRULENCE FACTORS THAT DAMAGE THE HOST

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response

a. Overall Mechanism

Fundamental Statements for this Softchalk Lesson:

***1.* In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms.**
2. 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.

3. PAMPS bind to pattern-recognition receptors (PRRs) on defense cells which lead to the production of cytokines that trigger inflammation, activate the complement pathways, and activate the coagulation pathway. This inflammatory response is accomplished primarily by an inflammatory programmed cell death called pyroptosis involving protein cellular complexes called inflammasomes.

4. Cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8) are known as inflammatory cytokines because they promote inflammation.
5. Inflammation is the means by which body defense cells and defense chemicals leave the blood and enter the tissue around an injured or infected site.
6. Vasodilation is a reversible opening of the junctional zones between endothelial cells of the blood vessels and results in increased blood vessel

permeability. This enables plasma, the liquid portion of the blood, to enter the surrounding tissue. Increased capillary permeability also enables white blood cells to squeeze out of the blood vessels and enter the tissue.
7. When there is a minor infection with few bacteria present, low levels of PAMPs are present. This leads to moderate cytokine production by defense cells and, in general, promotes body defense.
8. During severe systemic infections with large numbers of bacteria present, high levels of PAMPs are released resulting in excessive cytokine production by the defense cells and this can harm the body.
9. Perfusion refers to the delivery of nutrients and oxygen via arterial blood to a capillary bed in tissue.
10. Sepsis is an infection that leads to a systemic inflammatory response resulting in physiologic changes occurring at the capillary endothelial level. This systemic inflammatory response is referred to as Systemic Inflammatory Response Syndrome or SIRS.
11. Cytokine-induced extracellular killing by neutrophils adhere to capillary walls results in damage to the capillary walls and leakage of blood into surrounding tissue. This contributes to a decreased volume of circulating blood (hypovolemia).
12. Prolonged vasodilation and the resulting increased capillary permeability causes plasma to leave the bloodstream and enter the tissue. This contributes to a decreased volume of circulating blood (hypovolemia).
13. Prolonged vasodilation also leads to decreased vascular resistance within blood vessels resulting in a drop in blood pressure (hypotension).
14. At high levels of TNF, vascular smooth muscle tone and myocardial contractility are inhibited. This results in a marked hypotension.
15. Activation of the blood coagulation pathway can cause clots called microthrombi to form within the blood vessels throughout the body (disseminated intravascular coagulation or DIC). These microthrombi block the capillaries. Depletion of clotting factors leads to hemorrhaging in many parts of the body following neutrophil-induced capillary damage.
16. Increased capillary permeability as a result of vasodilation in the lungs, as well as neutrophil-induced injury to capillaries in the alveoli leads to acute inflammation, pulmonary edema, and loss of gas exchange in the lungs (acute respiratory distress syndrome or ARDS). As a result, the blood does not become oxygenated.
17. The combination of hypotension, hypovolemia, DIC, ARDS, results in hypoperfusion.
18. Without oxygen, cells switch to fermentation and produce lactic acid leading to hyperlactemia. This may then lower the pH of the blood (lactic acidosis). A blood pH range between 6.8 and 7.8 is needed for normal cellular enzyme activity in humans. Changes in the pH of arterial blood extracellular fluid outside this range lead to irreversible cell damage.
19. Collectively, this can result in end-organ ischemia (a restriction in blood supply that results in damage or dysfunction of tissues or organs), multiple system organ failure (MSOF), and death.
20. According to the NIH Sepsis Fact Sheet, "Every year, severe sepsis strikes about 750,000 Americans. It's been estimated that between 28 and 50 percent of these people die - far more than the number of U.S. deaths from prostate cancer, breast cancer and AIDS combined."

21. Approximately 45% of the cases of septicemia are due to Gram-positive bacteria, 45% are a result of Gram-negative bacteria, and 10% are due to fungi (mainly the yeast Candida).

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that may harm the host and give relevant examples.

4. Recall the mechanisms behind systemic inflammatory response syndrome and how this can be triggered by a bacterial infection.

5. Describe how and under what conditions what are usually normal innate and adaptive immune responses can harm the body.

Detailed Learning Objectives

1*. Define cytokine and chemokine and name 3 inflammatory cytokines.

2*. State the mechanism behind inflammation and state why it is primarily beneficial to the body.

3*. Briefly describe why inflammation during a minor or moderate infection is essentially beneficial while inflammation during a massive infection can cause considerable damage to the body.

4**. Looking at the overall mechanism behind septic shock, answer the following:

a. Describe how bacterial PAMPS initiate SIRS.

b. Define hypotension and describe the biological mechanism behind 2 factors that contribute to hypotension.

c. Define hypovolemia and describe the biological mechanism behind 3 factors that contribute to hypovolemia.

d. Define hypoperfusion and describe the biological mechanism behind 3 factors that contribute to hypoperfusion.

e. Describe the biological mechanism behind ARDS and how ARDS contributes to hypoperfusion.

f. Describe the sequence of events that enables hypoperfusion to lead to irreversible cell damage.

5*. Define pyroptosis and inflammasome and state their role in inducing inflammation.

6**. Define the following:

a. vasodilation
b. septicemia
c. hypotension
d. hypovolemia
e. septic shock
f. DIC
g. ARDS
h. MOSF
i. hypoperfusion

(*) = Common theme throughout the course

(**) = More depth and common theme

TPS Questions

In this section on Bacterial Pathogenesis we are looking at **virulence factors that damage the host**. Virulence factors that damage the host include:

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response.
2. The ability to produce harmful exotoxins.
3. The ability to induce autoimmune responses.

We will now look at the how the ability of PAMPs to trigger the production of inflammatory cytokines can sometimes result in an excessive inflammatory response.

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response

a. Overall Mechanism

PAMPs, PRRs, Cytokines, and Inflammation

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** (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.)

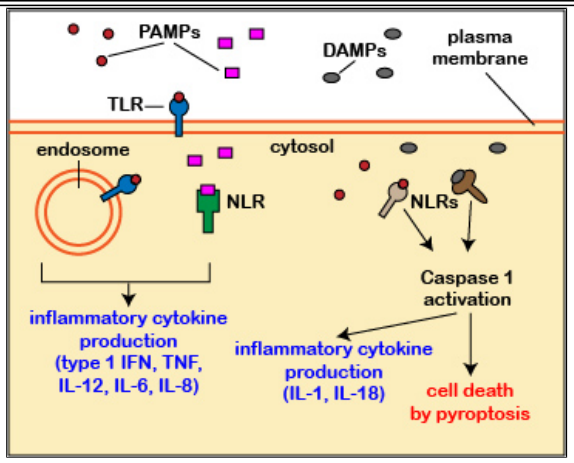
For more information: Preview of pathogen-associated molecular patterns (PAMPs)

Molecules such as peptidoglycan monomers, teichoic acids, LPS, porins, mycolic acid, arabinogalactin, flagellin, and mannose, are examples of bacterial PAMPs that bind to **pattern-recognition receptors (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, fever, and phagocytosis. This is accomplished primarily by an inflammatory programmed cell death called **pyroptosis** involving protein cellular complexes called **inflammasomes**.

Pyroptosis is a **programmed inflammatory death of host cells** that is mediated by an enzyme called caspase 1 and can be triggered by a variety of stimuli, including pathogen-associated molecular patterns (PAMPs) from microbial infections, as well as danger-associated molecular patterns (DAMPs) produced as a result of tissue injury during cancer, heart attack, and stroke. **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.

Pyroptosis is initiated by P**AMPs 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. 1)**. 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.

Fig. 1: 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.)

The binding of PAMPs to PRRs also leads to activation of the complement pathways and activation of the coagulation pathway.

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

Cytokines such as **tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), and interleukin-8 (IL-8)** are known as **inflammatory cytokines** because they **promote inflammation**. Some cytokines, such as IL-8, are also known as **chemokines**. Chemokines promote an inflammatory response by enabling white blood cells to leave the blood vessels and enter the surrounding tissue, by chemotactically attracting these white blood cells to the infection site, and by triggering neutrophils to release killing agents for extracellular killing.

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 defense chemicals leave the blood and enter the tissue around an injured or infected site**. The release of inflammatory cytokines eventually leads to vasodilation of blood vessels. **Vasodilation** is a reversible opening of the junctional zones between endothelial cells of the blood vessels and results in increased blood vessel permeability. This enables plasma, the liquid portion of the blood, to enter the surrounding tissue.The plasma contains defense chemicals such as antibody molecules, complement proteins, lysozyme, and human defensins. Increased capillary permeability also enables white blood cells to squeeze out of the blood vessels and enter the tissue. As can be seen, inflammation is necessary part of body defense. Excessive or prolonged inflammation can, however, cause harm as will be discussed below.

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.

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

Flash animation showing vasodilation.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing vasodilation.

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.

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Illustration of arterioles, venules, and a capillary bed.

As mentioned in a previous section, products of the **complement pathways** lead to: 1)more inflammation; 2) opsonization of bacteria; 3) chemotaxis of phagocytes to the infected site; and 4) MAC lysis of Gram-negative bacteria.

For more information: Preview of the complement pathways

The products of the **coagulation pathway** lead to the clotting of blood to stop bleeding, more inflammation, and localization of infection.

At moderate levels, inflammation, products of the complement pathways, and products of the coagulation pathway are essential to body defense. However, these same processes and products when excessive can cause considerable harm to the body.

Flash animation illustrating signaling toll-like receptors on defense cells: LPS and TLR-4.

html5 version of animation for iPad illustrating signaling toll-like receptors on defense cells: LPS and TLR-4.

1) Gram-negative bacteria release lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall.
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.
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:

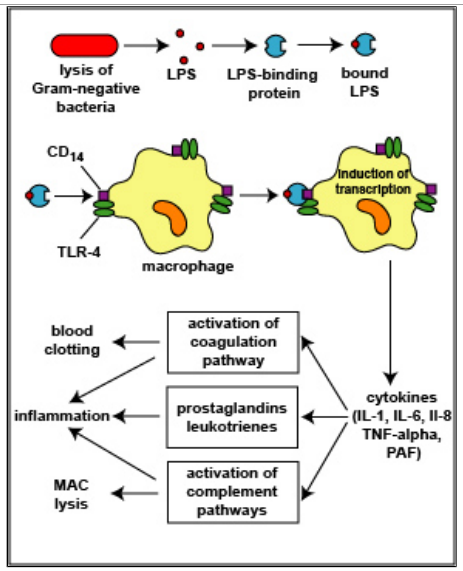
a. Interleukin-1 (IL-1) and Tumor necrosis factor-alpha (TNF-alpha): enhance inflammatory responses;
b. Interleukin-8 (IL-8): aids in the ability of white blood cells to leave the blood vessels and enter the tissue; a chemoattractant for phagocytes;
c. Interleukin-6 (IL-6) promotes B-lymphocyte activity;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

4) Cytokine genes are transcribed into mRNA molecules that goes to the cytoplasm to be translated into inflammatory cytokines that are subsequently secreted from the cell.

During **minor local infections** with few bacteria present, **low levels of cell wall PAMPs are released** leading to **moderate cytokine production** by defense cells such as monocytes, macrophages, and dendritic cells and, in general, **promoting body defense** by stimulating inflammation and moderate fever, breaking down energy reserves to supply energy for defense, activating the complement pathway and the coagulation pathway, and generally stimulating immune responses **(see Fig. 1)**. Also as a result of these cytokines, circulating phagocytic white blood cells such as neutrophils and monocytes stick to the walls of capillaries, squeeze out and enter the tissue, a process termed **diapedesis**. The phagocytic white blood cells such as neutrophils then kill the invading microbes with their proteases and toxic oxygen radicals. These defenses will be covered in greater detail in Units 5 and 6.

However, 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. 2)**. 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 (MSOF)**, and **death**.

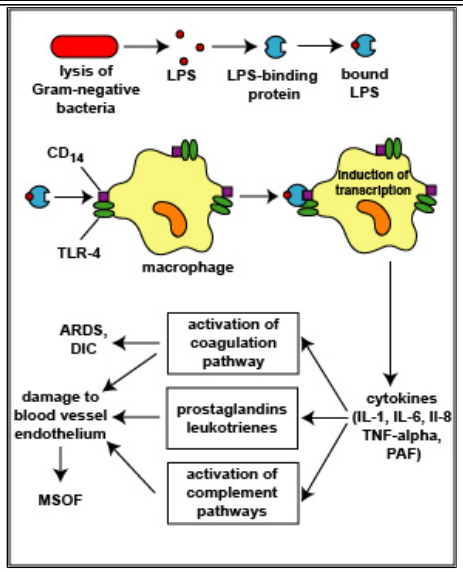
Fig. 1: Physiologic Action of Lipopolysaccharide (LPS) from the Gram-Negative Cell Wall



The lysis of Gram-negative bacteria causes them to release lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall. 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 TLR-4 to respond to the LPS, triggering the macrophage 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. LPS can also bind directly to TLR-4 molecules.

(LPS, lipopolysaccharide;TLR, toll-like receptor; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8, TNF-alpha, tumor necrosis factor-alpha; PAF, platelet-activating factor.) This will be discussed in greater detail under Bacterial Pathogenicity.

Fig. 2: Harmful Effects of Lipopolysaccharide (LPS; Endotoxin) Released from the Gram-Negative Cell Wall



The lysis of Gram-negative bacteria causes them to release lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall. 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 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 MSOF.

(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; MSOF, multiple system organ failure.)

Concept Map for Synthesizing and Secreting Inflammatory Cytokines and Chemokines in Response to PAMPs

Quiz Group

Sepsis and Systemic Inflammatory Response Syndrome (SIRS)

Keep in mind that a primary function of the circulatory system is perfusion, the delivery of nutrients and oxygen via arterial blood to a capillary bed in tissue. This, in turn, delivers nutrients for cellular metabolism and oxygen for energy production via aerobic respiration to all of the cells of the body.

Sepsis is an infection that leads to a systemic inflammatory response resulting in physiologic changes occurring at the capillary endothelial level. This systemic inflammatory response is referred to as **Systemic Inflammatory Response Syndrome or SIRS.**

Based on severity, there are three sepsis syndromes based on severity:

1. **Sepsis**. SIRS in the setting of an infection.

2. **Severe sepsis**. An infection with end-organ dysfunction as a result of hypoperfusion, the reduced delivery of nutrients and oxygen to tissues and organs via the blood.

3. **Septic shock**. Severe sepsis with persistent hypotension and tissue hypoperfusion despite fluid resuscitation.

We will now take a look at the underlying mechanism of SIRS that can result in septic shock.

Systemic Inflammatory Response Syndrome (SIRS) Resulting in Septic Shock

During a severe systemic infection, an excessive inflammatory response triggered by overproduction of inflammatory cytokines such as TNF-alpha, IL-1, IL-6, IL-8, and PAF in response to PAMPs often occurs.

The release of inflammatory cytokines eventually leads to vasodilation of blood vessels. **Vasodilation** is a reversible opening of the junctional zones between endothelial cells of the blood vessels and results in increased blood vessel permeability. Normally, this fights the infection by enabling plasma, the liquid portion of the blood, to enter the surrounding tissue.The plasma contains defense chemicals such as antibody molecules, complement proteins, lysozyme, and human defensins. Increased capillary permeability also enables white blood cells to adhere to the inner capillary wall, squeeze out of the blood vessels, and enter the tissue to fight infection, a process called diapedesis.

Excessive productions of cytokines during a systemic infection results in the following events:

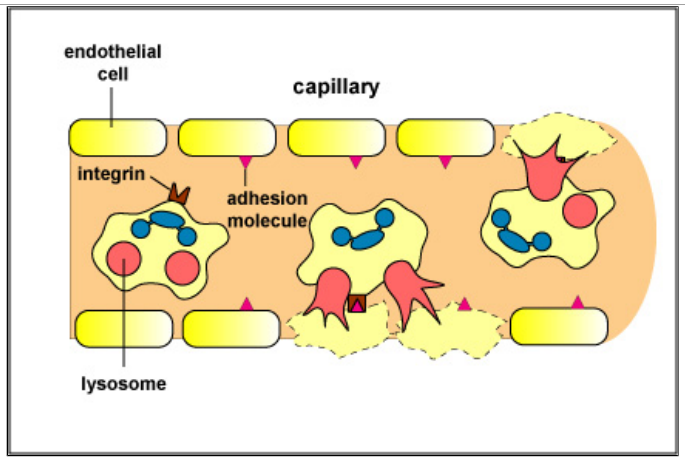
1. During diapedesis, phagocytic WBCs called **neutrophils adhere to capillary walls in massive amounts**.

   a. Chemokines such as IL-8 activate extracellular killing by neutrophils, causing them to **release proteases and toxic oxygen radicals** while still in the capillaries. These are the same toxic chemicals neutrophils use to kill microbes, but now they are dumped onto the vascular endothelial cells to which the neutrophils have adhered.

   b. These events result in **damage to the capillary walls and leakage of blood into surrounding tissue (see Fig. 3)**. This contributes to a **decreased volume of circulating blood (hypovolemia)**.

   c. Hypovolemia then contributes to hypoperfusion.

Fig. 3: Capillary Damage during Systemic Inflammatory Response Syndrome (SIRS)



With the production of large amounts of proinflammatory cytokines, neutrophils adhere to capillary walls in massive amounts. Chemokines cause neutrophils to release proteases and toxic oxygen radicals, the same chemicals they use to kill microbes, but these toxic chemicals are now being dumped onto the vascular endothelial cells to which the neutrophils have adhered during diapedesis. This results in damage to the capillary walls and leakage of blood.

Flash animation summarizing early inflammation and diapedesis.

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 up regulation 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 up regulates 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 are attracted towards chemotactic agents such as the complement protein C5a and leukotriene B_4 generated by cells at the site of infection or injury.

Flash animation summarizing late inflammation and diapedesis.

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 an maintains the inflammatory response by up regulation 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.

Flash animation of extracellular killing by neutrophils.
Copyright © Gary E. Kaiser
html5 version of animation for iPad of extracellular killing by neutrophils.
The binding of LPS released from the Gram-negative cell wall binds to toll-like receptor- 4 (TLR- 4), as well as the binding of chemokines such as interleukin-8 (IL-8) to their respective chemokine receptors on the surface of neutrophils stimulates the neutrophils to release proteases and toxic oxygen radicals for extracellular killing.

2. Prolonged vasodilation and the resulting increased capillary permeability causes plasma to leave the bloodstream and enter the tissue. Activation of the complement pathways and production of vasodilators such as C5a, C3a, prostaglandins, and leukotrienes further contributes to fluid loss.

a. This contributes to a **decreased volume of circulating blood (hypovolemia)**.

b. Hypovolemia then contributes to hypoperfusion.

Prolonged vasodilation also leads to **decreased vascular resistance** within blood vessels.

a. This, in turn, contributes to a **drop in blood pressure (hypotension)**.

b. Hypotension then contributes to hypoperfusion.

Flash animation showing vasodilation.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing vasodilation.
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.

3. At high levels of TNF, vascular smooth muscle tone and myocardial contractility are inhibited.

a. This results in a marked **hypotension**.

b. Hypotension then contributes to hypoperfusion.

Cytokine-induced overproduction of nitric oxide (NO) by cardiac muscle cells and vascular smooth muscle cells can also lead to heart failure.

4. Activation of the blood coagulation pathway can cause **clots called microthrombi to form within the blood vessels throughout the body.** This is called **disseminated intravascular coagulation (DIC)**.

a. These microthrombi block the capillaries and contribute to hypoperfusion.

b. Activation of neutrophils also leads to their accumulation and plugging of the vasculature.

c. Depletion of clotting factors as a result of DIC leads to hemorrhaging in many parts of the body following the neutrophil-induced capillary damage. This, as mentioned above, contributes to a decreased volume of circulating blood or hypovolemia.

d. Hypovolemia then contributes to hypotension.

e. Hypotension then contributes to hypoperfusion.

5. In the lungs, the increased capillary permeability as a result of vasodilation in the lungs, as well as neutrophil-induced **injury to capillaries in the alveoli** leads to **acute inflammation, pulmonary edema, and loss of gas exchange** in the lungs. This condition is called **acute respiratory distress syndrome (ARDS)**.

a. As a result, the blood does not become oxygenated.

b. Lack of oxygenation of the blood via the lungs then causes hypoperfusion.

6. In the liver, hypoperfusion and capillary damage results in impaired liver function and a failure to maintain normal blood glucose levels.

Overuse of glucose by muscles and a failure of the liver to replace glucose can lead to a **drop in blood glucose level** below what is needed to sustain life.

(Glucose is needed to make ATP via aerobic respiration.)

7. Hypoperfusion can also leads to kidney and bowel injury.

8. The combination of hypotension, hypovolemia, DIC, ARDS, and the resulting hypoperfusion may lead to lactic acidosis.

a. Without oxygen, cells switch to fermentation and produce lactic acid (hyperlactemia) that, if not removed by the liver and kidneys, may lower the pH of the blood (lactic acidosis). A blood pH range between 6.8 and 7.8 is needed for normal cellular enzyme activity in humans.

b. Changes in the pH of arterial blood extracellular fluid outside this range lead to irreversible cell damage.

In summary, the release of excessive levels of inflammatory cytokines in response to PAMPs binding to PRRs during a systemic infection results in:

1. **A drop in blood volume or hypovolemia**. This is caused by the following events:

a. **Extracellular killing by neutrophils damages the capillary walls** resulting in **blood and plasma leaving the bloodstream and entering the surrounding tissue**.

b. **Depletion of clotting factors during disseminated intravascular coagulation (DIC) can lead to hemorrhaging** as the capillaries are damaged.

c. **Prolonged vasodilation** results in **plasma leaving the bloodstream and entering the surrounding tissue**.

2. **A drop in blood pressure or hypotension**. This is a result of the following events:

a. **Prolonged vasodilation causes decreased vascular resistance within blood vessels decreases blood pressur**e.

b. **High levels of TNF, inhibit vascular smooth muscle tone and myocardial contractility decreasing the ability of the heart to pump blood** throughout the body.

c. **Hypovolemia from capillary damage, plasma leakage, and hemorrhaging**.

3. T**he inability to deliver nutrients and oxygen to body cells or hypoperfusion**. This is a result of the following events:

a. **Activation of the blood coagulation pathway can cause clots called microthrombi to form within the blood vessels throughout the body causing disseminated intravascular coagulation (DIC)** which **blocks the flow of blood through the capillaries** and, as mentioned above, depletion of clotting factors can lead to **hemorrhaging** in many parts of the body.

b. **Increased capillary permeability as a result of vasodilation in the lungs**, as well as **neutrophil-induced injury to capillaries in the alveoli leads to acute inflammation, pulmonary edema, and loss of gas exchange in the lungs (acute respiratory distress syndrome or ARDS)**. As a result, the **blood does not become oxygenated**.

c. **Hypovolemia decreases the volume of circulating blood and leads to hypotension**.

d. **Hypotension decreases the pressure needed to deliver blood throughout the body**.

6. **Hypoperfusion in the liver** can result in a **drop in blood glucose level from liver dysfunction**. Glucose is needed for ATP production during glycolysis and aerobic respiration. A drop in glucose levels can result in **decreased ATP production and insufficient energy for cellular metabolism**.

7. **The lack of oxygen delivery as a result of hypoperfusion causes cells to switch to fermentation for energy production**. The **lactic acid end products of fermentation may lead to lactic acidosis and a blood pH to low for the functioning of the enzymes involved in cellular metabolism**. This can result in **irreversible cell death**.

Collectively, this can result in :

- **end-organ ischemia** Ischemia is a restriction in blood supply that results in damage or dysfunction of tissues or organs.
- **multiple system organ failure (MSOF)**.
- **death.**

For more on SIRS and Septic Shock, see Septic Shock.

Concept map for SIRS and Septic Shock

TPS Questions

Septicemia is a condition where bacteria enter the blood and cause harm. According to the NIH *Sepsis Fact Sheet*, "Every year, severe sepsis strikes about 750,000 Americans. It's been estimated that between 28 and 50 percent of these people die - far more than the number of U.S. deaths from prostate cancer, breast cancer and AIDS combined." Factors contributing to this high rate of sepsis include:

1. An aging US population.
2. Increased longevity of people with chronic diseases.
3. An increase in number of invasive medical procedures performed.
4. Increased use of immunosuppressive and chemotherapeutic agents.
5. The spread of antibiotic-resistant microorganisms.

People that survive severe sepsis may have permanent damage to the lungs or other organs. Approximately 45% of the cases of septicemia are due to Gram-positive bacteria, 45% are a result of Gram-negative bacteria, and 10% are due to fungi (mainly the yeast *Candida*). Many of these cases of septicemia are **healthcare-associated infections (HAIs)**.

The Centers for Disease Control and Prevention (CDC) Healthcare-associated infection's website reports that "In American hospitals alone, healthcare-associated infections account for an estimated 1.7 million infections and 99,000 associated deaths each year. Of these infections:

- 32 percent of all healthcare-associated infection are urinary tract infections
- 22 percent are surgical site infections
- 15 percent are pneumonia (lung infections)
- 14 percent are bloodstream infections"

Estimates of Healthcare-Associated Infections (HCIs) 2011

from CDC

We will now look at various bacterial cell wall components that lead to cytokine production, inflammation, and activation of the complement and coagulation pathways.

Self Quiz for the Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response: a. Overall Mechanism

Quiz Group

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Back to Softchalk Lessons Table of Contents

PAMPs in Gram-negative bacteria
VIRULENCE FACTORS THAT DAMAGE THE HOST

C. VIRULENCE FACTORS THAT DAMAGE THE HOST

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response

b. Gram-Negative PAMPs: LPS (Endotoxin), Porins in the Outer Membrane, Peptidoglycan Monomers, Mannose-Rich Glycans, and Flagellin

Fundamental Statements for this Softchalk Lesson:

1. PAMPs associated with Gram-negative bacteria include LPS (endotoxin) and porins in the outer membrane, peptidoglycan fragments, mannose-rich sugars, and flagellin.
2. Approximately 45% of the cases of septicemia are due to Gram-negative bacteria.
3. Medically important Gram-negative bacteria include such classical pathogens as Neisseria meningitidis, Salmonella, Neisseria gonorrhoeae, and Hemophilus influenzae type b.
4. Many normal Gram negative intestinal microbiota such as Escherichia coli, Proteus, Klebsiella, Enterobacter, Serratia, and Pseudomonas aeruginosa are responsible for a variety of opportunistic infections including urinary tract infections, wound infections, pneumonia, and septicemia.
5. The four most common Gram-negative bacteria causing Healthcare-associated infections (HAIs) are Escherichia coli, Pseudomonas aeruginosa, Enterobacter species, and Klebsiella pneumoniae. Collectively, these four bacteria accounted for 32% of all nosocomial infections in the U.S. between 1990 and 1996. There are over two million HAIs per year in the U.S.

Common Course Objectives

1. Recall the factors that influence disease severity.
2. Explain how diseases can be transmitted.
3. Describe virulence factors that may harm the host and give relevant examples.
4. Recall the mechanisms behind systemic inflammatory response syndrome and how this can be triggered by a bacterial infection.
5. Describe how and under what conditions what are usually normal innate and adaptive immune responses can harm the body.
6. Determine the difference between endotoxin and exotoxin

Detailed Learning Objectives

1. State what is meant by endotoxin and indicate where it is normally found.
2*. List 3 Gram-negative PAMPS and briefly describe how they initiate SIRS.
3*. Define healthcare-associated infection (HAI) and name 3 common Gram-negative bacteria that cause HAIs.

(*) = Common theme throughout the course

Highlighted Bacterium

1. Read the description of *Pseudomonas aeruginosa* and match the bacterium with the description of the organism and the infection it causes.

TPS Questions

In this section on Bacterial Pathogenesis we are looking at **virulence factors that damage the host**. Virulence factors that damage the host include:

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response.
2. The ability to produce harmful exotoxins.
3. The ability to induce autoimmune responses.

We will now look at the ability of Gram-negative bacteria to produce PAMPs that bind to host cells and cause them to synthesize and secrete inflammatory cytokines.

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response

b. Gram-Negative PAMPs: LPS (Endotoxin), Porins in the Outer Membrane, Peptidoglycan Monomers, Mannose-Rich Glycans, and Flagellin

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** (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.)

Molecules unique to bacteria, such as peptidoglycan monomers, teichoic acids, LPS, porins, mycolic acid, mannose-rich glycans, and flagellin, are PAMPs that bind to **pattern-recognition receptors** (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, fever, and phagocytosis. This is accomplished primarily by an inflammatory programmed cell death called **pyroptosis** involving protein cellular complexes called **inflammasomes**.

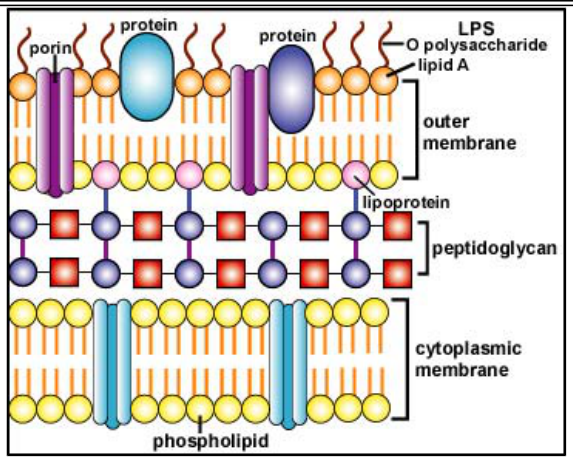
Pyroptosis is a **programmed inflammatory death of host cells** that is mediated by an enzyme called caspase 1 and can be triggered by a variety of stimuli, including pathogen-associated molecular patterns (PAMPs) from microbial infections, as well as danger-associated molecular patterns (DAMPs) produced as a result

of tissue injury during cancer, heart attack, and stroke. **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. The binding of PAMPs to PRRs also leads to activation of the complement pathways and activation of the coagulation pathway.

Cytokines such as **tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), and interleukin-8 (IL-8)** are known as **inflammatory cytokines** because they **promote inflammation**. Some cytokines, such as IL-8, are also known as **chemokines**. Chemokines promote an inflammatory response by enabling white blood cells to leave the blood vessels and enter the surrounding tissue, by chemotactically attracting these white blood cells to the infection site, and by triggering neutrophils to release killing agents for extracellular killing.

As mentioned in Unit 1, the lipopolysaccharide (LPS) in the outer membrane of the Gram-negative cell wall is also known as **endotoxin**. While porins, mannose-rich glycans, peptidoglycan fragments, and flagellin also function as PAMPs, the most significant Gram-negative-associated PAMP is LPS. Gram-negative bacteria release some endotoxin during their normal replication but endotoxin is released in quantity upon death and degradation of the bacterium. The degree of damage from endotoxin is related to the degree of release of the LPS from the bacterium's cell wall.

Fig. 1: Illustration of the Structure of a Gram-Negative Cell Wall



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.

For more Information: Review of the Gram-negative cell wall

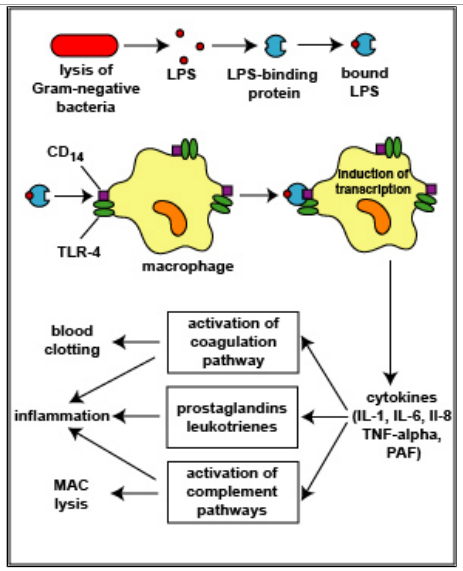
1. The **LPS** released from the outer membrane of the Gram-negative cell wall typically **binds first to a LPS-binding protein** circulating in the blood and this complex, in turn, **binds to a receptor molecule called CD14 that is found on the surface of defense cells such as macrophages and dendritic cells (see Fig. 2)** located in most tissues and organs of the body.

2. The interaction of the LPS-binding protein with CD14 is thought to **promote the ability of the toll-like receptor TLR-4 to respond to the LPS**.

3. The interaction between LPS and its TLRs triggers the macrophage to release various defense regulatory chemicals called cytokines, including **tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8), and platelet-activating factor (PAF) (see Fig. 2)**. The cytokines then bind to cytokine receptors on target cells and initiate an inflammatory response. They also activate both the complement pathways and the coagulation pathway **(see Fig. 2)**.

4. The binding of of LPS molecules to their TLRs on the surfaces of phagocytic white blood cells called **neutrophils** causes them to **release proteases** and **toxic oxygen radicals** for extracellular killing. Chemokines such as interleukin-8 (IL-8) also stimulate extracellular killing. In addition, LPS and cytokines stimulate the synthesis of a vasodilator called **nitric oxide**.

Fig. 2: Physiologic Action of Lipopolysaccharide (LPS) from the Gram-Negative Cell Wall



The lysis of Gram-negative bacteria causes them to release lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall. 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 TLR-4 to respond to the LPS, triggering the macrophage 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. LPS can also bind directly to TLR-4 molecules. (LPS, lipopolysaccharide;TLR, toll-like receptor; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8, TNF-alpha, tumor necrosis factor-alpha; PAF, platelet-activating factor.) This will be discussed in greater detail under Bacterial Pathogenicity.

Flash animation illustrating signaling toll-like receptors on defense cells: LPS and TLR-4.

html version of animation for iPAD illustrating extracellular killing by neutrophils triggered by the binding of LPS and chemokines to receptors on neutrophils.

1) Gram-negative bacteria release lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall.
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.
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:

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)

4) Cytokine genes are transcribed into mRNA molecules that goes to the cytoplasm to be translated into inflammatory cytokines that are subsequently secreted from the cell.

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

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

For more information: Preview of cytokines

Flash animation of extracellular killing by neutrophils triggered by the binding of LPS and chemokines to receptors on neutrophils.

html version of animation for iPAD illustrating extracellular killing by neutrophils triggered by the binding of LPS and chemokines to receptors on neutrophils.

The binding of LPS released from the gram-negative cell wall binds to toll-like receptor- 4 (TLR- 4), as well as the binding of chemokines such as interleukin-8 (IL-8) to their respective chemokine receptors on the surface of neutrophils stimulates the neutrophils to release proteases and toxic oxygen radicals for extracellular killing.

During **minor local infections** with few bacteria present, **low levels of Gram-negative PAMPs are released** leading to **moderate cytokine production** by defense cells such as monocytes, macrophages, and dendritic cells and, in general, **promoting body defense** by stimulating inflammation and moderate fever, breaking down energy reserves to supply energy for defense, activating the complement pathway and the coagulation pathway, and generally stimulating immune responses (see Fig. 1). Also as a result of these cytokines, circulating phagocytic white blood cells such as neutrophils and monocytes stick to the walls of capillaries, squeeze out and enter the tissue, a process termed **diapedesis**. The phagocytic white blood cells such as neutrophils then kill the invading microbes with their proteases and toxic oxygen radicals. These defenses will be covered in greater detail in Units 5 and 6.

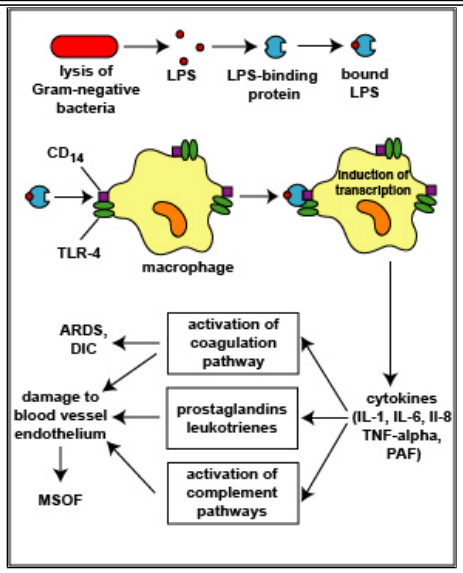
For more information: Preview of inflammation

For more information: Preview of the complement pathways

However, during **severe systemic infections** with large numbers of bacteria present, **high levels of Gram-negative PAMPs are released** resulting in **excessive cytokine production** by the defense cells and this can **harm the body (see Fig. 3)**. 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 (MSOF), and often death.

Fig. 3: Harmful Effects of Lipopolysaccharide (LPS; Endotoxin) Released from the Gram-Negative Cell Wall



The lysis of Gram-negative bacteria causes them to release lipopolysaccharide (LPS; endotoxin) from the outer membrane of their cell wall. 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 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 MSOF. (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; MSOF, multiple system organ failure.)

TPS Questions

As seen earlier in this unit, **the release of excessive levels of inflammatory cytokines in response to PAMPs binding to PRRs during a systemic infection results in**:

1. **A drop in blood volume or hypovolemia**. This is caused by the following events:

   a. **Extracellular killing by neutrophils damages the capillary walls** results in **blood and plasma leaving the bloodstream and entering the surrounding tissue**.

   b. **Depletion of clotting factors during disseminated intravascular coagulation (DIC) can lead to hemorrhaging** as the capillaries are damaged.

   c. **Prolonged vasodilation** results in **plasma leaving the bloodstream and entering the surrounding tissue**.

2. **A drop in blood pressure or hypotension**. This is a result of the following events:

   a. **Prolonged vasodilation causes decreased vascular resistance within blood vessels decreases blood pressur**e.

   b. **High levels of TNF, inhibit vascular smooth muscle tone and myocardial contractility decreasing the ability of the heart to pump blood** throughout the body.

   c. **Hypovolemia from capillary damage, plasma leakage, and hemorrhaging**.

3. T**he inability to deliver nutrients and oxygen to body cells or hypoperfusion**. This is a result of the following events:

   a. **Activation of the blood coagulation pathway can cause clots called microthrombi to form within the blood vessels throughout the body causing disseminated intravascular coagulation (DIC)** which **blocks the flow of blood through the capillaries** and, as mentioned above, depletion of clotting factors can lead to **hemorrhaging** in many parts of the body.

   b. **Increased capillary permeability as a result of vasodilation in the lungs**, as well as **neutrophil-induced injury to capillaries in the alveoli leads to acute inflammation, pulmonary edema, and loss of gas exchange in the lungs (acute respiratory distress syndrome or ARDS)**. As a result, the **blood does not become oxygenated**.

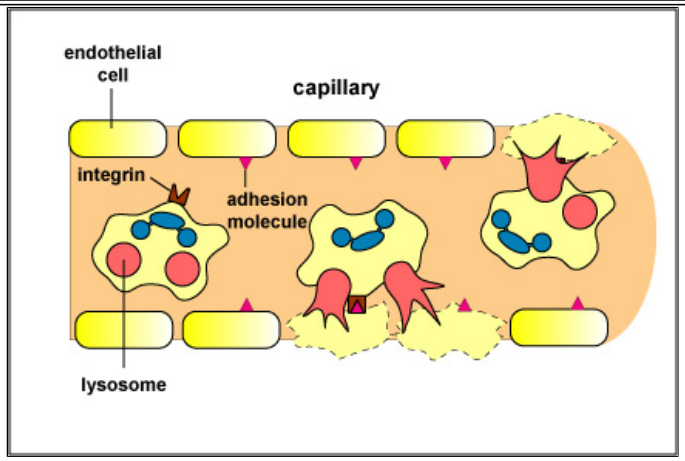
   c. **Hypovolemia decreases the volume of circulating blood and leads to hypotension**.

   d. **Hypotension decreases the pressure needed to deliver blood throughout the body**.

6. **Hypoperfusion in the liver** can result in a **drop in blood glucose level from liver dysfunction**. Glucose is needed for ATP production during glycolysis and aerobic respiration. A drop in glucose levels can result in **decreased ATP production and insufficient energy for cellular metabolism**.

7. **The lack of oxygen delivery as a result of hypoperfusion causes cells to switch to fermentation for energy production**. The **lactic acid end products of fermentation lead to lactic acidosis and the wrong pH for the functioning of the enzymes involved in cellular metabolism**. This can result in **irreversible cell death**.

Fig. 4: Capillary Damage during Systemic Inflammatory Response Syndrome (SIRS)



With the production of large amounts of proinflammatory cytokines, neutrophils adhere to capillary walls in massive amounts. Chemokines cause neutrophils to release proteases and toxic oxygen radicals, the same chemicals they use to kill microbes, but these toxic chemicals are now being dumped onto the vascular endothelial cells to which the neutrophils have adhered during diapedesis. This results in damage to the capillary walls and leakage of blood.

Collectively, this can result in:

- end-organ ischemia Ischemia is a restriction in blood supply that results in damage or dysfunction of tissues or organs.
- multiple system organ failure (MSOF).
- death.

For more information: Review of SIRS and septic shock

Concept Map for Synthesizing and Secreting Inflammatory Cytokines and Chemokines in Response to PAMP

Concept Map for SIRS and Septic Shock

Septicemia is a condition where bacteria enter the blood and cause harm. According to the NIH *Sepsis Fact Sheet*, "Every year, severe sepsis strikes about 750,000 Americans. It's been estimated that between 28 and 50 percent of these people die - far more than the number of U.S. deaths from prostate cancer, breast cancer and AIDS combined." Factors contributing to this high rate of sepsis include:

1. An aging US population.
2. Increased longevity of people with chronic diseases.
3. An increase in number of invasive medical procedures performed.
4. Increased use of immunosuppressive and chemotherapeutic agents.
5. The spread of antibiotic-resistant microorganisms.

People that survive severe sepsis may have permanent damage to the lungs or other organs. Approximately 45% of the cases of septicemia are due to Gram-positive bacteria, 45% are a result of Gram-negative bacteria, and 10% are due to fungi (mainly the yeast *Candida*). Many of these cases of septicemia are healthcare-associated infections (HAIs).

Other examples of damage from Gram-negative PAMPs are Gram-negative **bacterial meningitis** and pneumonia. The same inflammatory events lead to identical effects in the brain and the decreased delivery of oxygen and glucose to the cells of the brain results in damage and death of brain tissue. When Gram-negative bacteria enter the alveoli of the lungs and are lysed by antibiotics or body defenses, Gram-negative bacterial PAMPs bind to receptors on endothelial cells, the alveolar epithelium, and leukocytes causing the release of TNF-alpha, Il-1, and chemokines. **This leads to increased vascular permeability that enables serous fluids, red blood cells, and leukocytes to enter the air spaces of the lung where gas exchange occurs**. This prevents normal gas exchange and the person drowns on his or her own serous fluids.

Medically important Gram-negative bacteria include such classical pathogens as *Neisseria meningitidis*, *Salmonella*, *Neisseria gonorrhoeae*, and *Hemophilus influenzae* type b.

In addition, many normal Gram negative intestinal microbiota such as *Escherichia coli, Proteus, Klebsiella, Enterobacter*, *Serratia, and Pseudomonas aeruginosa* are responsible for a variety of **opportunistic infections** including urinary tract infections, wound infections, pneumonia, and septicemia. These bacteria owe much of their damage to LPS.

Highlighted Bacterium: Pseudomonas aeruginosa

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

These normal flora Gram-negative bacilli (along with Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis*) are among the most common causes of **healthcare-associated infections (HAIs)**. The four most common Gram-negative bacteria causing HCIs are *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* species, and *Klebsiella pneumoniae*. Collectively, these four bacteria accounted for 32% of all nosocomial infections in the U.S. between 1990 and 1996. There are over two million HAIs per year in the U.S.

According to the Centers for Disease Control and Prevention (CDC) Healthcare-associated infection's website, "In American hospitals alone, healthcare-associated infections account for an estimated 1.7 million infections and 99,000 associated deaths each year. Of these infections:

- 32 percent of all healthcare-associated infection are urinary tract infections
- 22 percent are surgical site infections
- 15 percent are pneumonia (lung infections)
- 14 percent are bloodstream infections"

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Salmonella* species
- *Escherichia coli*
- *Proteus* species
- *Klebsiella* species
- *Enterobacter* species
- *Serratia* species
- *Pseudomonas aeruginosa*

Self Quiz for Gram-Negative PAMPs: LPS (Endotoxin), Porins in the Outer Membrane, Peptidoglycan Monomers, Mannose-Rich Glycans, and Flagellin

Quiz Group

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Back to Softchalk Lessons Table of Contents

PAMPs in Gram-positive bacteria
VIRULENCE FACTORS THAT DAMAGE THE HOST

C. VIRULENCE FACTORS THAT DAMAGE THE HOST

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response

c. Gram-Positive PAMPs: Lipoteichoic Acids, Peptidoglycan Monomers, Mannose-Rich Glycans, and Flagellin

Fundamental Statements for this Softchalk Lesson:

1. ***PAMPs associated with Gram-positive bacteria include cell wall teichoic and lipotechoic acids, peptidoglycan fragments, mannose-rich sugars, and flagellin.***
2. ***Approximately 45% of the cases of septicemia are due to Gram-positive bacteria.***
3. ***Medically important Gram-positive bacteria include Staphylococcus aureus, coagulase-negative staphylococci, Enterococcus species, and Streptococcus pneumoniae.***
4. ***The three most common Gram-positive bacteria causing healthcare-associated infections (HAIs) are Staphylococcus aureus, coagulase-negative staphylococci, and Enterococcus species. Collectively, these three bacteria accounted for 34% of all HAIs in the U.S. between 1990 and 1996. There are over two million HAIs per year in the U.S.***

Common Course Objectives

1. Recall the factors that influence disease severity.
2. Explain how diseases can be transmitted.
3. Describe virulence factors that may harm the host and give relevant examples.
4. Recall the mechanisms behind systemic inflammatory response syndrome and how this can be triggered by a bacterial infection.
5. Describe how and under what conditions what are usually normal innate and adaptive immune responses can harm the body.

Detailed Learning Objectives

1*. Describe how Gram-positive PAMPS initiate SIRS.

2*. Name 2 Gram-positive bacteria that commonly cause healthcare-associated infections (HAIs).

(*) = Common theme throughout the course

Highlighted Bacterium

1. Read the description of *Staphylococcus aureus* and match the bacterium with the description of the organism and the infection it causes.

In this section on Bacterial Pathogenesis we are looking at **virulence factors that damage the host**. Virulence factors that damage the host include:

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response.
2. The ability to produce harmful exotoxins.
3. The ability to induce autoimmune responses.

We will now look at the ability of Gram-positive bacteria to produce PAMPs that bind to host cells and cause them to synthesize and secrete inflammatory cytokines.

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response

c. Gram-Positive PAMPs: Lipoteichoic Acids, Peptidoglycan Monomers, Mannose-Rich Glycans, and Flagellin

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** (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.)

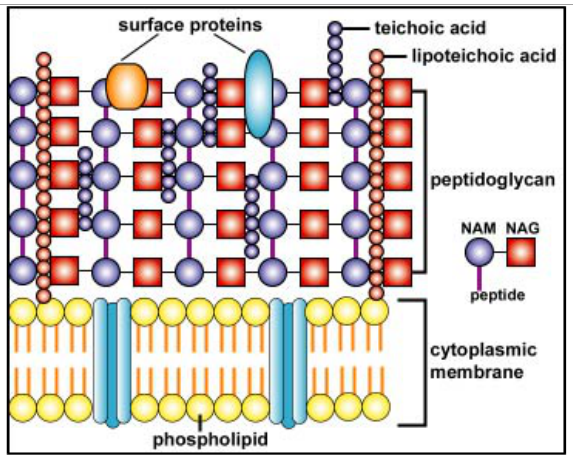
Molecules unique to bacteria, such as peptidoglycan monomers, teichoic acids, LPS, porins, mycolic acid, mannose-rich glycans, and flagellin, are PAMPs that bind to **pattern-recognition receptors** (PRRs) on a variety of defense cells of the body causing them to **synthesize and secrete a variety of proteins called cytokines**. These cytokines can, in turn **promote innate immune defenses** such as inflammation, fever, and phagocytosis. This is accomplished primarily by an inflammatory programmed cell death called **pyroptosis** involving protein cellular complexes called **inflammasomes**.

Pyroptosis is a **programmed inflammatory death of host cells** that is mediated by an enzyme called caspase 1 and can be triggered by a variety of stimuli, including pathogen-associated molecular patterns (PAMPs) from microbial infections, as well as danger-associated molecular patterns (DAMPs) produced as a result of tissue injury during cancer, heart attack, and stroke. **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. The binding of PAMPs to PRRs also leads to activation of the complement pathways and activation of the coagulation pathway.

Cytokines such as **tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), and interleukin-8 (IL-8)** are known as **inflammatory cytokines** because they **promote inflammation**. Some cytokines, such as IL-8, are also known as **chemokines**. Chemokines promote an inflammatory response by enabling white blood cells to leave the blood vessels and enter the surrounding tissue, by chemotactically attracting these white blood cells to the infection site, and by triggering neutrophils to release killing agents for extracellular killing.

PAMPs in Gram-positive bacteria include mannose-rich glycans, teichoic and lipoteichoic acids, peptidoglycan fragments **(see Fig. 1)**, and flagellin.

Fig. 1: Illustration of the Structure of a Gram-Positive Cell Wall



The Gram-positive cell wall appears as dense layer typically composed of numerous rows of peptidoglycan, and molecules of lipoteichoic acid, wall teichoic acid and surface proteins.

For more Information: Review of the Gram-positive cell wall

The mechanism is as follows:

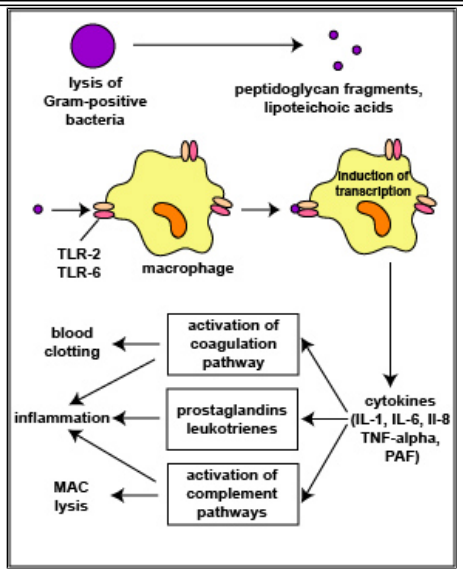
1. The lysis of Gram-positive bacteria causes PAMPs such as **peptidoglycan monomers (the building blocks of peptidoglycan (see Fig. 2), lipotechoic acids,** mannose-rich glycans, and flagellin to be released.

2. These PAMPs, in turn, bind to pattern-recognition receptors (PRRs) that are specific for these PAMPs that are found on the surface of body defense cells such as macrophages and dendritic cells.

3. Binding of the PAMPs to the PRRs of these defense cells triggers them to release various defense regulatory chemicals called cytokines, including **tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), inflammatory chemokines such as IL-8, and platelet-activating factor (PAF) (see Fig. 2)**. The cytokines then bind to cytokine receptors on target cells and initiate an inflammatory response. They also activate both the complement pathways and the coagulation pathway **(see Fig. 2)**, in a manner similar to endotoxin (LPS) from the Gram-negative cell wall.

4. The binding of PAMPs to their PRRs on the surfaces of phagocytic white blood cells called **neutrophils** causes them to **release proteases** and **toxic oxygen radicals** for extracellular killing. Chemokines such as interleukin-8 (IL-8) also stimulate extracellular killing. In addition, cytokines stimulate the synthesis of a vasodilator called **nitric oxide**.

Fig. 2: Physiologic Action of PAMPs from the Gram-Positive Cell Wall



The lysis of Gram-positive bacteria causes them to release peptidoglycan fragments and lipoteichoic acids from their cell wall. These cell wall components, in turn, bind to toll-like receptors such as TLR-2 and TLR-6 that are specific for these cell wall components and are 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 and initiate inflammation and activate both the complement pathways and the coagulation pathway. These gram-positive cell wall components can also bind first to binding proteins circulating in the blood that subsequently carry them to CD14 molecules on the macrophages. (TLR, toll-like receptor; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8, TNF-alpha, tumor necrosis factor-alpha; PAF, platelet-activating factor; MAC, membrane attack complex.)

Flash animation illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6.

html5 version of animation for iPad illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6.

1) Gram-positive bacteria release lipoteichoic acid (LTA) from their cell wall.
2) The LTA binds to a TLR-2/TLR-6 pair on defense cells such as macrophages and dendritic cells.
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:

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)

4) Cytokine genes are transcribed into mRNA molecules that goes to the cytoplasm to be translated into inflammatory cytokines that are subsequently secreted from the cell.

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

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

For more information: Preview of cytokines

Flash animation showing the binding of teichoic acid and chemokines to receptors on neutrophils and their subsequent release of killing agents.

html5 version of animation for iPad showing the binding of teichoic acid and chemokines to receptors on neutrophils and their subsequent release of killing agents.

The binding of teichoic acids (TA) released from the gram-positive cell wall binds to toll-like receptor- 2,6 pairs (TLR- 2,6), as well as the binding of chemokines such as interleukin-8 (IL-8) to their respective chemokine receptors on the surface of neutrophils stimulates the neutrophils to release proteases and toxic oxygen radicals for extracellular killing.

During **minor local infections** with few bacteria present, **low levels of Gram-positive PAMPs are released** leading to **moderate cytokine production** by defense cells such as monocytes, macrophages, and dendritic cells and, in general, **promoting body defense** by stimulating inflammation and moderate fever, breaking down energy reserves to supply energy for defense, activating the complement pathway and the coagulation pathway, and generally stimulating immune responses **(see Fig. 2)**. Also as a result of these cytokines, circulating phagocytic white blood cells such as neutrophils and monocytes stick to the walls of capillaries, squeeze out and enter the tissue, a process termed **diapedesis**. The phagocytic white blood cells such as neutrophils then kill the invading microbes with their proteases and toxic oxygen radicals. These defenses will be covered in greater detail in Units 5 and 6.

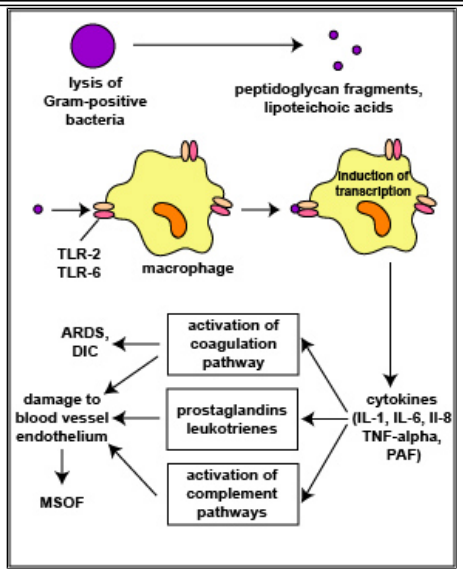
For more information: Preview of inflammation

For more information: Preview of the complement pathways

However, during **severe systemic infections** with large numbers of bacteria present, **high levels of Gram-positive PAMPs are released** resulting in **excessive cytokine production** by the defense cells and this can **harm the body (see Fig. 3)**. 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 (MSOF), and often death.

Fig. 3: The Harmful Effects of Peptidoglycan Fragments and Lipoteichoic Acid Released During Gram-Positive Infections



The lysis of Gram-positive bacteria causes them to release peptidoglycan fragments and lipoteichoic acids from their cell wall. These cell wall components, in turn, bind to toll-like receptors such as TLR-2 and TLR-6 that are specific for these cell wall components and are 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 MSOF. (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; MSOF, multiplesystem organ failure.)

As seen earlier in this unit, **the release of excessive levels of inflammatory cytokines in response to PAMPs binding to PRRs during a systemic infection results in**:

1. **A drop in blood volume or hypovolemia**. This is caused by the following events:

   a. **Extracellular killing by neutrophils damages the capillary walls** results in **blood and plasma leaving the bloodstream and entering the surrounding tissue**.

   b. **Depletion of clotting factors during disseminated intravascular coagulation (DIC) can lead to hemorrhaging** as the capillaries are damaged.

   c. **Prolonged vasodilation** results in **plasma leaving the bloodstream and entering the surrounding tissue**.

2. **A drop in blood pressure or hypotension**. This is a result of the following events:

   a. **Prolonged vasodilation causes decreased vascular resistance within blood vessels decreases blood pressur**e.

   b. **High levels of TNF, inhibit vascular smooth muscle tone and myocardial contractility decreasing the ability of the heart to pump blood** throughout the body.

   c. **Hypovolemia from capillary damage, plasma leakage, and hemorrhaging**.

3. T**he inability to deliver nutrients and oxygen to body cells or hypoperfusion**. This is a result of the following events:

a. **Activation of the blood coagulation pathway can cause clots called microthrombi to form within the blood vessels throughout the body causing disseminated intravascular coagulation (DIC)** which **blocks the flow of blood through the capillaries** and, as mentioned above, depletion of clotting factors can lead to **hemorrhaging** in many parts of the body.

b. **Increased capillary permeability as a result of vasodilation in the lungs**, as well as **neutrophil-induced injury to capillaries in the alveoli leads to acute inflammation, pulmonary edema, and loss of gas exchange in the lungs (acute respiratory distress syndrome or ARDS)**. As a result, the **blood does not become oxygenated**.

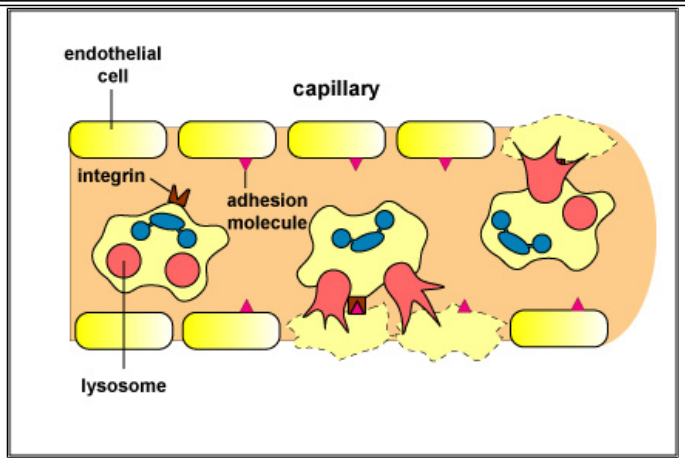
c. **Hypovolemia decreases the volume of circulating blood and leads to hypotension**.

d. **Hypotension decreases the pressure needed to deliver blood throughout the body**.

6. **Hypoperfusion in the liver** can result in a **drop in blood glucose level from liver dysfunction**. Glucose is needed for ATP production during glycolysis and aerobic respiration. A drop in glucose levels can result in **decreased ATP production and insufficient energy for cellular metabolism**.

7. **The lack of oxygen delivery as a result of hypoperfusion causes cells to switch to fermentation for energy production**. The **lactic acid end products of fermentation lead to lactic acidosis and the wrong pH for the functioning of the enzymes involved in cellular metabolism**. This can result in **irreversible cell death**.

Fig. 4: Capillary Damage during Systemic Inflammatory Response Syndrome (SIRS)



With the production of large amounts of proinflammatory cytokines, neutrophils adhere to capillary walls in massive amounts. Chemokines cause neutrophils to release proteases and toxic oxygen radicals, the same chemicals they use to kill microbes, but these toxic chemicals are now being dumped onto the vascular endothelial cells to which the neutrophils have adhered during diapedesis. This results in damage to the capillary walls and leakage of blood.

Collectively, this can result in:

- end-organ ischemia Ischemia is a restriction in blood supply that results in damage or dysfunction of tissues or organs.
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For more information: Review of SIRS and septic shock
Concept Map for Synthesizing and Secreting Inflammatory Cytokines and Chemokines in Response to PAMP
Concept Map for SIRS and Septic Shock

Septicemia is a condition where bacteria enter the blood and cause harm. According to the NIH *Sepsis Fact Sheet*, "Every year, severe sepsis strikes about 750,000 Americans. It's been estimated that between 28 and 50 percent of these people die - far more than the number of U.S. deaths from prostate cancer, breast cancer and AIDS combined." Factors contributing to this high rate of sepsis include:

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2. Increased longevity of people with chronic diseases.
3. An increase in number of invasive medical procedures performed.

4. Increased use of immunosuppressive and chemotherapeutic agents.
5. The spread of antibiotic-resistant microorganisms.

People that survive severe sepsis may have permanent damage to the lungs or other organs. Approximately 45% of the cases of septicemia are due to Gram-positive bacteria, 45% are a result of Gram-negative bacteria, and 10% are due to fungi (mainly the yeast *Candida*). Many of these cases of septicemia are healthcare-associated infections (HAIs).

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. *Staphylococcus aureus*, coagulase-negative staphylococci, and *Enterococcus* species are among the leading Gram-positive bacteria to cause septicemia.

Other examples of damage from Gram-positive PAMPs are Gram-positive **bacterial meningitis** and pneumonia. The same inflammatory events lead to identical effects in the brain and the decreased delivery of oxygen and glucose to the cells of the brain results in damage and death of brain tissue.

One such example is the pneumococcus, *Streptococcus pneumoniae*. When *S. pneumoniae* enters the alveoli of the lungs and is lysed by antibiotics or body defenses, glycopeptide cell wall fragments and techoic acids bind to receptors on endothelial cells, the alveolar epithelium, and leukocytes causing the release of TNF-alpha, Il-1, and chemokines. **This leads to increased vascular permeability that enables serous fluids, red blood cells, and leukocytes to enter the air spaces of the lung where gas exchange occurs**. This prevents normal gas exchange and the person drowns on his or her own serous fluids. From the lungs, *S. pneumoniae* often invades the blood, crosses the blood-brain barrier, and enters the meninges.

Gram-positive bacteria such as *Staphylococcus* and *Enterococcus*, along with the normal microbiota Gram-negative bacteria mentioned in the previous section, are among the most common causes of **healthcare-associated infections (HAIs)**. The three most common Gram-positive bacteria causing HAIs are *Staphylococcus aureus*, coagulase-negative staphylococci, and *Enterococcus* species. Collectively, these three bacteria accounted for 34% of all HAIs in the U.S. between 1990 and 1996. There are over two million HAIs per year in the U.S.

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- 22 percent are surgical site infections
- 15 percent are pneumonia (lung infections)
- 14 percent are bloodstream infections"

Highlighted Bacterium: *Staphylococcus aureus*

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

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Streptococcus pneumoniae*
- *Staphylococcus* species
- *Enterococcus* species

Self Quiz for Gram-Positive PAMPs: Lipoteichoic Acids, Peptidoglycan Monomers, Mannose-Rich Glycans, and Flagellin

Quiz Group

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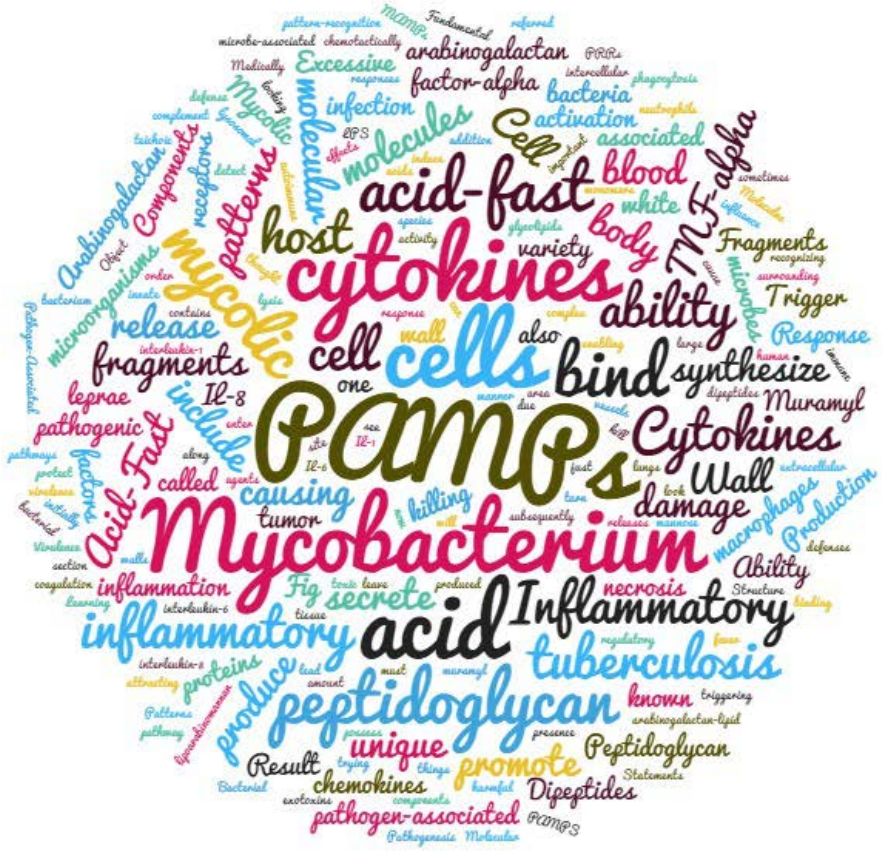
PAMPs in acid-fast bacteria
VIRULENCE FACTORS THAT DAMAGE THE HOST

C. VIRULENCE FACTORS THAT DAMAGE THE HOST

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response

d. Acid-Fast Bacterial PAMPs: Mycolic Acid, Arabinogalactan, and Peptidoglycan Fragments (Muramyl Dipeptides)



Fundamental Statements for this Softchalk Lesson:

1. PAMPs associated with acid-fast bacteria include mycolic acid, arabinogalactan, and peptidoglycan fragments.
2. Medically important acid-fast bacterium include Mycobacterium tuberculosis* and *Mycobacterium leprae.

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that may harm the host and give relevant examples.
4. Recall the mechanisms behind systemic inflammatory response syndrome and how this can be triggered by a bacterial infection.
5. Describe how and under what conditions what are usually normal innate and adaptive immune responses can harm the body.

Detailed Learning Objectives

1. Name the common PAMPs associated with acid-fast bacteria that stimulate cytokine production and an inflammatory response.
2. Name pathogenic 2 acid-fast bacteria and state the infection each causes.

In this section on Bacterial Pathogenesis we are looking at **virulence factors that damage the host**. Virulence factors that damage the host include:

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response.
2. The ability to produce harmful exotoxins.
3. The ability to induce autoimmune responses.

We will now look at the ability of acid-fast bacteria to produce PAMPs that bind to host cells and cause them to synthesize and secrete inflammatory cytokines.

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response

d. Acid-Fast Bacterial PAMPs: Mycolic Acid, Arabinogalactan, and Peptidoglycan Fragments (Muramyl Dipeptides)

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** (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.)

Molecules unique to bacteria, such as peptidoglycan monomers, teichoic acids, LPS, porins, mycolic acid, mannose-rich glycans, and flagellin, are PAMPs that bind to **pattern-recognition receptors** (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, fever, and phagocytosis. The binding of PAMPs to PRRs also leads to activation of the complement pathways and activation of the coagulation pathway.

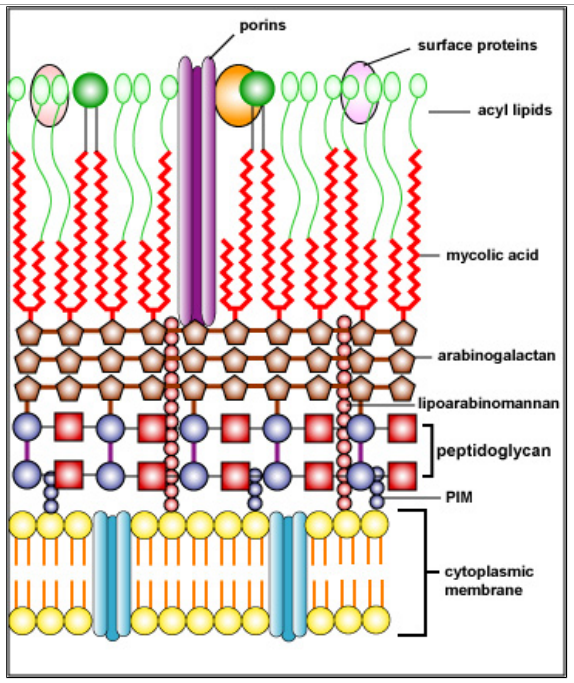
Cytokines such as **tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), and interleukin-8 (IL-8)** are known as **inflammatory cytokines** because they **promote inflammation**. Some cytokines, such as IL-8, are also known as **chemokines**. Chemokines promote an inflammatory response by enabling white blood cells to leave the blood vessels and enter the surrounding tissue, by chemotactically attracting these white blood cells to the infection site, and by triggering neutrophils to release killing agents for extracellular killing.

For more information: Review of the acid-fast cell wall

Concept Map for Synthesizing and Secreting Inflammatory Cytokines and Chemokines in Response to PAMPs

The lysis of pathogenic *Mycobacterium* species, such as *Mycobacterium tuberculosis* and *Mycobacterium leprae,* releases mycolic acid, arabinogalactan, and peptidoglycan fragments (muramyl dipeptides) from their acid-fast cell wall **(see Fig. 1)**. The **mycolic acid molecules, arabinogalactan, and peptidoglycan fragments bind to pattern-recognition receptors on macrophages** and dendritic cells **causing them to release cytokines such as tumor necrosis factor-alpha (TNF-alpha)**. Most of the damage in the lungs during tuberculosis is thought to be due to the effects TNF-alpha along with the release of toxic lysosomal components of the macrophages trying to kill the *Mycobacterium tuberculosis*.

Fig .1: Illustration of the 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 phosphatidyinositol 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.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Mycobacterium tuberculosis*
- *Mycobacterium leprae*
- *Mycobacterium avium-intracellulare* complex

Self Quiz for Acid-Fast Bacterial PAMPs: Mycolic Acid, Arabinogalactan, and Peptidoglycan Fragments (Muramyl Dipeptides)

Quiz Group

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An overview of bacterial exotoxins
VIRULENCE FACTORS THAT DAMAGE THE HOST

C. VIRULENCE FACTORS THAT DAMAGE THE HOST

1. The Ability to Produce Harmful Exotoxins

An Overview

Fundamental Statements for this Softchalk Lesson:

1. Exotoxins are toxins, often protein in nature, secreted from a living bacterium.
2. Some bacteria use various secretion systems to inject toxins directly into human cells.
3. There are three main types of exotoxins: superantigens (type I toxins); exotoxins that damage host cell membranes (type II toxins); and A-B toxins and other toxin that interfere with host cell function (type III toxins).
4. The body's major defense against exotoxins is the production of antitoxin antibodies. Once the antibody binds to the exotoxin, the toxin can no longer bind to the receptors on the host cell membrane.

Common Course Objectives

1. Recall the factors that influence disease severity.
2. Explain how diseases can be transmitted.
3. Describe virulence factors that may harm the host and give relevant examples.
4. Determine the difference between endotoxin and exotoxin.
5. Discuss the significance of exotoxins and recognize specific examples.
6. Discuss how exoenzymes aid in the development of disease.

Detailed Learning Objectives

1. Define exotoxin and list three types of exotoxins.
2*. State the major way the body defends itself against exotoxins.

(*) = Common theme throughout the course

In this section on Bacterial Pathogenesis we are looking at **virulence factors that damage the host**. Virulence factors that damage the host include:

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response.
2. The ability to produce harmful exotoxins.

3. The ability to induce autoimmune responses.

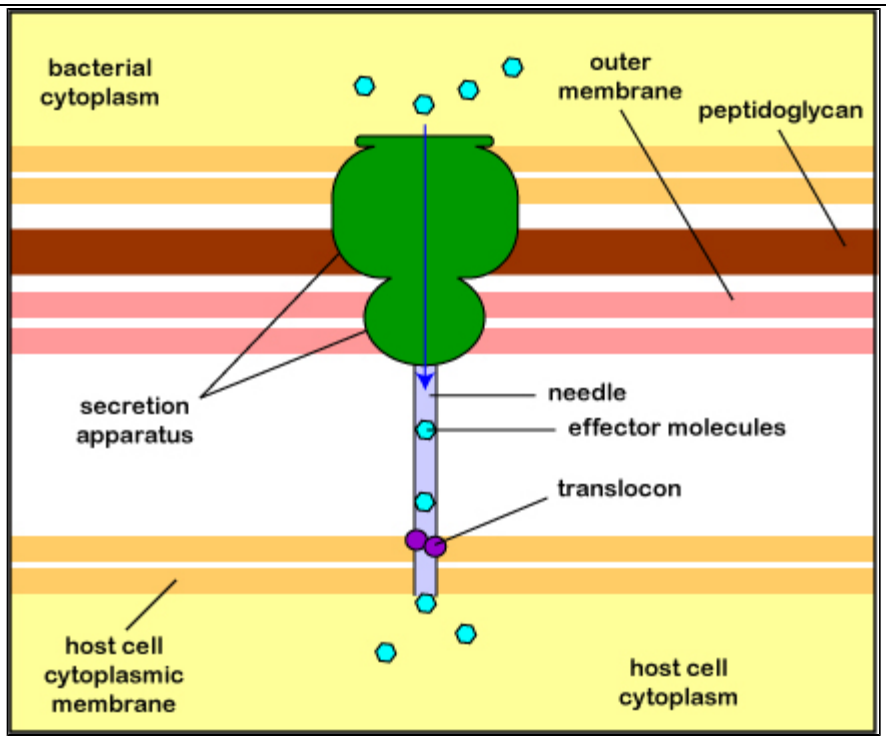
We will now look at the ability of bacteria to produce harmful exotoxins.

1. The Ability to Produce Harmful Exotoxins

An Overview

Exotoxins are **toxins**, often proteins in nature, **secreted from a living bacterium** but also **released upon bacterial lysis**. In addition, some bacteria use various secretion systems such as the type 3 secretion system **(see Fig. 1)** to **inject toxins directly into human cells**. (As learned earlier, the lipopolysaccharide or LPS portion of the Gram-negative bacterial cell wall is known as endotoxin, a PAMP that can initiate an excessive inflammatory response in the host. It was originally called endotoxin because it was located within the Gram-negative cell wall as opposed to being secreted from bacteria as in the case of exotoxins.)

Fig. 1: The Bacterial Type 3 Secretion System



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.

Not all exotoxins are necessarily produced to harm humans. Some may be designed to play a role in bacterial physiology, such as resisting bacteriophages, regulating cellular function, or quorum sensing. Other toxins may be produced primarily to target protozoa, insects, and smaller animals and harming human cells becomes an accidental side effect.

There are three main types of exotoxins:

1. **superantigens (Type I toxins)**;
2. **exotoxins that damage host cell membranes (Type II toxins)**; and
3. **A-B toxins and other toxin that interfere with host cell function (Type III toxins)**.

The body's major **defense against exotoxins** is the production of **antitoxin antibodies**. Once the antibody binds to the exotoxin, the toxin can no longer bind to the receptors on the host cell membrane.

Flash animation showing the neutralization of exotoxins with antibodies.
html5 version of animation for iPad showing the neutralization of exotoxins with antibodies.
The Fab portion of the antibodies made against epitopes of the binding site of an exotoxin blocks the exotoxin from binding to the host cell membrane. As a result, the toxin can not enter the cell and cause harm.

We will now look at each of these three types of exotoxins.

Self Quiz for the Ability to Produce Harmful Exotoxins: An Overview

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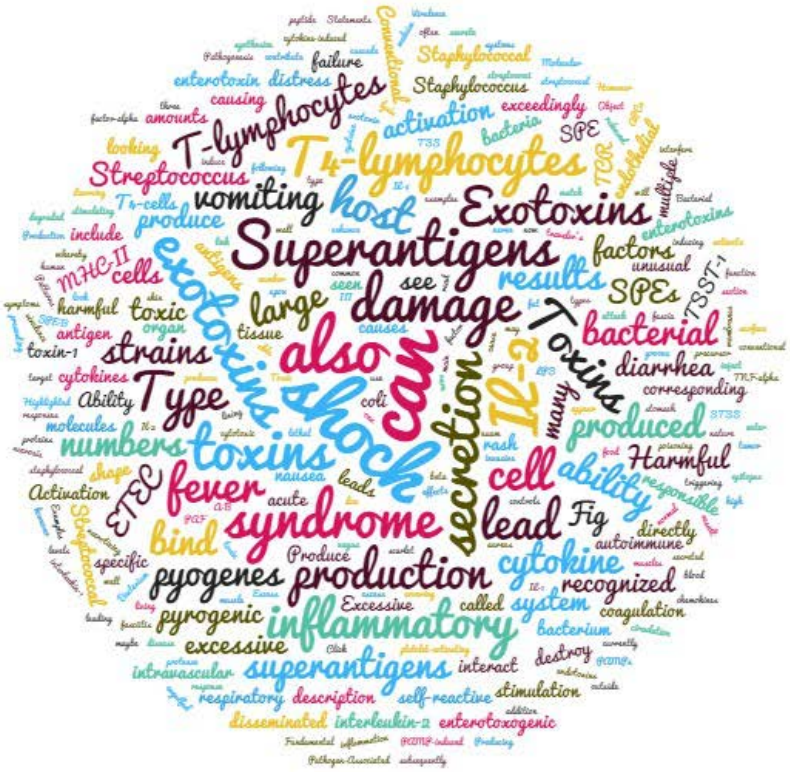
Type I bacterial toxins
VIRULENCE FACTORS THAT DAMAGE THE HOST

C. VIRULENCE FACTORS THAT DAMAGE THE HOST

2. The Ability to Produce Harmful Exotoxins

a. Type I toxins (Superantigens)



Fundamental Statements for this Softchalk Lesson:

1. Conventional antigens are only recognized by specific T4-cells having a TCR with a corresponding shape.
2. Superantigens are unusual bacterial toxins that interact with exceedingly large numbers of T4-lymphocytes.
3. Activation of very large numbers of T4-lymphocytes results in the secretion of excessive amounts of a cytokine called interleukin-2 (IL-2).
4. Excess stimulation of IL-2 secretion can also lead to production of inflammatory and can lead to the same endothelial damage, acute respiratory distress syndrome, disseminated intravascular coagulation, shock, and multiple organ system failure seen with PAMP-induced inflammation.
5. Examples of superantigens include toxic shock syndrome toxin-1 (TSST-1), Streptococcal pyrogenic exotoxins (SPE), Staphylococcal enterotoxins (SE), and enterotoxogenic E. coli (ETEC) enterotoxin.

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that may harm the host and give relevant examples.
4. Determine the difference between endotoxin and exotoxin.
5. Discuss the significance of exotoxins and recognize specific examples.
6. Discuss how exoenzymes aid in the development of disease.

Detailed Learning Objectives

1*. Define superantigen.

2. Briefly describe the mechanism by which superantigens cause harm to the body.

3. Name 2 superantigens and give an example of a bacterium that produces each.

(*) = Common theme throughout the course

Highlighted Bacterium

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

TPS Questions

In this section on Bacterial Pathogenesis we are looking at **virulence factors that damage the host**. Virulence factors that damage the host include:

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response.
2. The ability to produce harmful exotoxins.
3. The ability to induce autoimmune responses.

We will now look at the ability of bacteria to produce type I exotoxins.

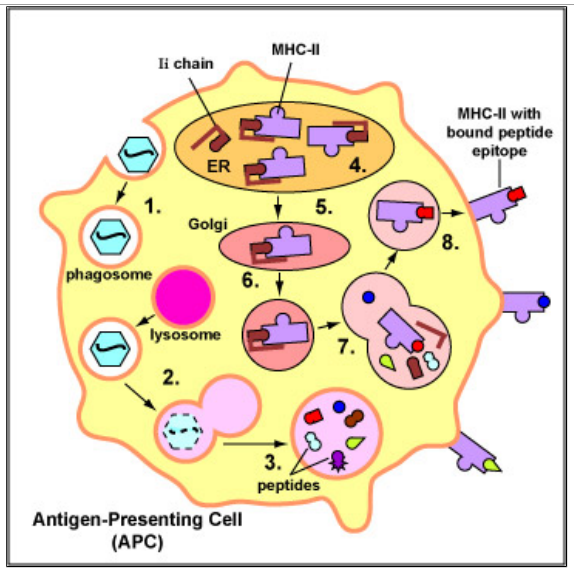
2. The Ability to Produce Harmful Exotoxins

a. Type I toxins (Superantigens)

Superantigens are unusual bacterial toxins that **interact with exceedingly large numbers of T4-lymphocytes**. They bind to the surface of the target cell but do not enter the cell.

Conventional antigens are engulfed by antigen presenting cells (APCs), degraded into epitopes, bind to the peptide groove of MHC-II molecules, and are put on the surface of the APC **(see Fig. 1)**. Here they are **recognized by specific T4-lymphocytes having a TCR with a corresponding shape (see Fig. 2)**.

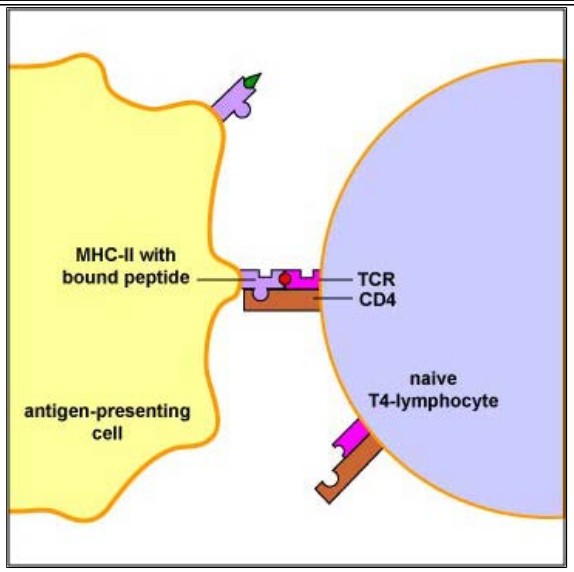
Fig. 1: Binding of Peptide Epitopes from Exogenous Antigens to MHC-II Molecules



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.

1. Exogenous antigens, such as viruses, are engulfed and placed in a phagosome.
2. Lysosomes fuse with the phagosome forming an 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 invarient 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.

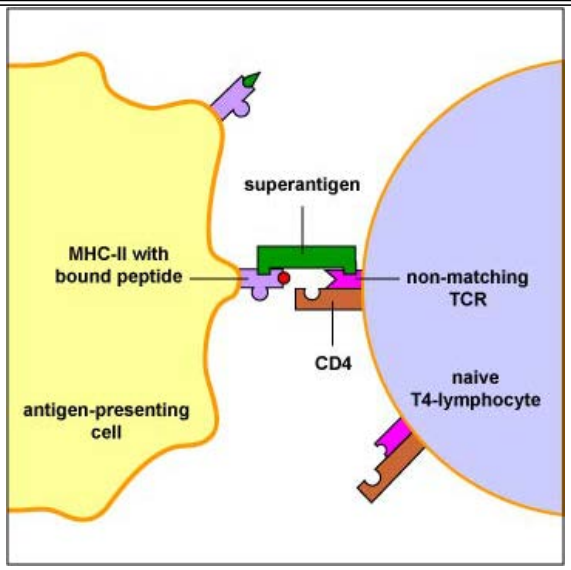
Fig. 2: Binding of T4-Lymphocytes to Conventional Antigens



Conventional antigens are only recognized by specific T4-lymphocytes having a TCR with a shape that corresponds to a peptide of that antigen bound to MHC-II molecules.

Superantigens, however, **bind directly to the outside of MHC-II molecules and activate large numbers of T4-lymphocytes (see Fig. 3)**. This activation of very large numbers of T4-lymphocytes results in the **secretion of excessive amounts of a cytokine called interleukin-2 (IL-2)** as well as the activation of self-reactive T-lymphocytes. The normal response to a conventional antigen results in the activation of maybe 1 in 10,000 T-lymphocytes; superantigens can activate as many as 1 in 5 T-lymphocytes.

Fig. 3: Binding of Superantigens



Conventional antigens are only recognized by specific T4-lymphocytes having a TCR with a shape that corresponds to a peptide of that antigen bound to MHC-II molecules. Superantigens, on the other hand, bind directly to the outside of MHC-II molecules and the TCRs and activate many T4-lymphocytes. A specific TCR is not required for activation.

Production of high levels of IL-2 can result in circulation of IL-2 in the blood leading to symptoms such as fever, nausea, vomiting, diarrhea, and malaise. However, **excess stimulation of IL-2 secretion can also lead to production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), inflammatory chemokines such as IL-8, and platelet-activating factor (PAF)**, and can lead to the same endothelial damage, acute respiratory distress syndrome, disseminated intravascular coagulation, shock, and multiple organ system failure seen above with LPS and other bacterial cell wall factors. Activation of self-reactive T-lymphocytes can also lead to autoimmune attack.

For more information: Review of the shock cascade

For more information: Preview of inflammation

The following are examples of superantigens.

1. Toxic shock syndrome toxin-1 (TSST-1), produced by some strains of *Staphylococcus aureus.* This exotoxin causes toxic shock syndrome (TSS). Excessive cytokine production leads to fever, rash, and shock.

2. Streptococcal pyrogenic exotoxin (Spe), produced by rare invasive strains and scarlet fever strains of *Streptococcus pyogenes* (the group A beta streptococci). *S pyogenes* produces a number of SPEs that are cytotoxic, pyrogenic, enhance the lethal effects of endotoxins, and contribute to cytokine-induced inflammatory damage. SPEs are responsible for causing streptococcal toxic shock syndrome (STSS) whereby excessive cytokine production leads to fever, rash, and triggering the shock cascade. The SPEs also appear to be responsible for inducing necrotizing fasciitis, a disease that can destroy the skin, fat, and tissue covering the muscle (the fascia). SPE B is also a precursor for a cysteine protease that can destroy muscles tissue.

3. Staphylococcal enterotoxins (SE), produced by many strains of *Staphylococcus aureus.* These exotoxins cause staphylococcal food poisoning. Excessive Il-2 production results in fever, nausea, vomiting,and diarrhea. The vomiting may also be due to these toxins stimulating the vagus nerve in the stomach lining that controls vomiting.

4. ETEC enterotoxin, produced by enterotoxogenic *E. coli* (ETEC), one of the most common causes of traveler's diarrhea.

Highlighted Bacterium: Streptococcus pyogenes

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

TPS Questions

Concept Map for Type I Toxins (Superantigens)

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Staphylococcus aureus* and *Streptococcus pyogenes*: Toxic shock syndrome
- *Staphylococcus aureus*: Staphylococcal food poisoning
- Enterotoxogenic *Escherichia coli* (ETEC)

Self Quiz for the Ability to Produce Harmful Exotoxins: Type I Toxins

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Type II bacterial toxins
VIRULENCE FACTORS THAT DAMAGE THE HOST

C. VIRULENCE FACTORS THAT DAMAGE THE HOST

2. The Ability to Produce Harmful Exotoxins

b. Type II toxins (Toxins that Damage Host Cell Membranes)

Fundamental Statements for this Softchalk Lesson:

1. Type II toxins are typically phospholipases or pore-forming cytotoxins that disrupt the integrity of eukaryotic cell membranes.
2. Damages host cells release danger-associated molecular patterns (DAMPs) that bind to pattern-recognition receptors (PRRs) causing the release of inflammatory cytokines. This inflammatory response can also further contribute to tissue damage.
3. Examples include the exotoxins of Clostridium perfringens that cause gas gangrene, exotoxins of Pseudomonas aeruginosa that causes a variety of opportunistic infections, exotoxins of Streptococcus pyogenes that causes strep throat, the exotoxins of Clostridium difficile that causes antibiotic-associated colitis, and leukotoxins, pore-forming toxins that causes lysis of white blood cells.

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that may harm the host and give relevant examples.

4. Determine the difference between endotoxin and exotoxin.
5. Discuss the significance of exotoxins and recognize specific examples.
6. Discuss how exoenzymes aid in the development of disease.

Detailed Learning Objectives

1. Briefly describe the roles of alpha toxin, kappa toxin, and mu toxin, and fermentation by *Clostridium perfringens* in the pathogenesis of gas gangrene.
2. State how the following toxins cause harm and name a bacterium producing each:
    a. leukotoxins such as leukocidin
    b. *Bordetella* tracheal cytotoxin
3. State how Toxin A and Toxin B of *Clostridium difficile* lead to diarrhea and damage to the colon.

Highlighted Bacterium

1. Read the description of *Clostridium difficile* and match the bacterium with the description of the organism and the infection it causes.

TPS Questions

In this section on Bacterial Pathogenesis we are looking at **virulence factors that damage the host**. Virulence factors that damage the host include:

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response.
2. The ability to produce harmful exotoxins.
3. The ability to induce autoimmune responses.

We will now look at the ability of bacteria to produce type II exotoxins.

2. The Ability to Produce Harmful Exotoxins

b. Type II toxins (Toxins that Damage Host Cell Membranes)

Type II toxins are typically phospholipases or pore-forming cytotoxins that disrupt the integrity of eukaryotic cell membranes. Damages host cells release danger-associated molecular patterns (DAMPs) that bind to pattern-recognition receptors (PRRs) causing the release of inflammatory cytokines. This inflammatory response can also further contribute to tissue damage.

1. The **exotoxins** of *Clostridium perfringens*. This bacterium produces at least 20 exotoxins that play a role in the pathogenesis of gas gangrene and producing expanding zones of dead tissue (necrosis) surrounding the bacteria. Toxins include:

- **alpha toxin** (lecithinase): **increases the permeability of capillaries and muscle cells by breaking down lecithin in cytoplasmic membranes**. This results in the gross **edema** of gas gangrene. If the alpha toxin enters the blood it can damage organs. Alpha toxin is also necrotizing, hemolytic, and cardiotoxic.
- **kappa toxin** (collagenase): **breaks down supportive connective tissue** resulting in the mushy lesions of gas gangrene. It is also necrotizing.
- **mu toxin** (hyaluronidase): **breaks down the tissue cement** that holds cells together in tissue.
- epsilon toxin: increases vascular permeability and causes edema and congestion in various organs including lungs and kidneys.
- Additional necrotizing toxins include beta toxin, iota toxin, and nu toxin.

A major characteristic of gas gangrene is the ability of *C. perfringens* to **very rapidly spread** from the initial wound site leaving behind an expanding zone of dead tissue. This organism spreads as a result of the pressure from fluid accumulation (due to increased capillary permeability from **alpha toxin**) and **gas production** (**anaerobic fermentation of glucose** by the organisms produces hydrogen and carbon dioxide), coupled with the breakdown of surrounding connective tissue (**kappa toxin**) and tissue cement (**mu toxin**).

2. **Leukotoxins.** Leukotoxins, such as leukocidin, are pore-forming toxins that **cause lysis of white blood cells** and other cells involved in immunity by binding to chemokine receptors on these cells and damaging the cell membrane. Leukotoxins are produced by various pyogenic bacteria including *Staphylococcus aureus* and *Streptococcus pyogenes*, (group A beta streptococci).

3. *Pseudomonas aeruginosa* produces a variety of toxins that lead to cell lysis and tissue damage in the host. Type II toxins include:

- Exotoxin U (Exo U): Degrades the plasma membrane of eukaryotic cells, leading to lysis.
- Phospholipase C (PLC): Damages cellular phospholipids causing tissue damage; stimulates inflammation. Delivered by a type 3 secretion system.
- Alkaline protease: leads to tissue damage.
- Cytotoxin: Damages cell membranes of leukocytes causes microvascular damage.
- Elastase: Destroys elastin, a protein that is a component of lung tissue.
- Pyocyanin: a green to blue water-soluble pigment that catalyzes the formation of tissue-damaging toxic oxygen radicales; impairs ciliary function, stimulates inflammation.

4. **Toxin A** and **Toxin B**, produced by *Clostridium difficile*. **Toxin A damages the membranes of intestinal mucosal cells** causing hypersecretion of fluids. In addition, it triggers the production of inflammatory cytokines. Finally, it also **attracts and destroys neutrophils**, causing them to release their lysosomal enzymes for further tissue damage leading to hemorrhagic necrosis. **Toxin B** depolymerizes actin **damaging the cytoskeleton of mucosal cells.** *C. difficile* causes severe **antibiotic-associated colitis** and is an opportunistic Gram-positive, endospore-producing bacillus transmitted by the fecal-oral route. *C. difficile* is a common healthcare-associated infection (HAIs) and is the most frequent cause of health-care-associated diarrhea.

Highlighted Bacterium: Clostridium difficile
Click on this link, read the description of *Clostridium difficile*, and be able to match the bacterium with its description on an exam.

5. *Streptococcus pyogenes* produces a number of enzymes and toxins that damage cells and tissues and **causes inflammation**:

- **Streptolysin S** : Causes lysis of red blood cell membranes.
- **Streptolysin O**: Lytic to cells that contain cholesterol in their plasma membrane.
- **Proteases**: Degrade cellular proteins;helps organism spread.
- **DNases**: Degrade cellular DNA; helps organism spread.
- **Streptokinase**: Breaks down fibrin in clots; helps organism spread.
- Streptococcal pyrogenic exotoxin B (SPE B): A protease that facilitates bacterial spreading and survival; induces inflammation during *S. pyogenes* infections.

6. Urease and phospholipase, produced by *Helicobacter pylori*. Urease contributes to acid resistance and epithelial cell damage while phospholipase damages the membrane of gastric or intestinal mucosal cells.

Flash animation showing induction of stomach and intestinal ulcers by *Helicobacter pylori*.
Copyright © Gary E. Kaiser
html5 version of animation for iPad showing induction of stomach and intestinal ulcers by *Helicobacter pylori*.
Helicobacter pylori, by means of its flagella, is able to swim through the mucus layer of the stomach or intestines and adhere to the epithelial cells of the mucous membranes. Here the pH is near neutral. To also help protect the bacterium from the acid, *H. pylori* produces an acid-inhibitory protein that blocks acid secretion by surrounding parietal cells in the stomach. The bacterium then releases toxins that lead to excessive production of cytokines and chemokines, as well as mucinase and phospholipase that damage the gastric or intestinal mucosa. The cytokines and chemokines, in turn, result in a massive inflammatory response. Neutrophils leave the capillaries, accumulate at the area of infection, and discharge their lysosomes for extracellular killing. This not only kills the bacteria, it also destroys the mucus-secreting mucous membranes of the stomach. Without this protective layer, gastric acid causes ulceration of the stomach or intestines. (Note that *H. pylori* is actually a spiral-shaped bacteria with a lophotrichous arrangement of flagella but showing this in the animation is beyond my technical abilities.)

7. Bordetella **tracheal cytotoxin**, produced by *Bordetella pertussis*. *Bordetella* tracheal cytotoxin causes the respiratory cell damage during whooping cough. Cell death, inhibition of ciliary movement by ciliated epithelial cells, and release of the inflammatory cytokine IL-1 triggers the violent coughing episodes, the only way the body can now remove inflammatory debris, bacteria, and mucus.

As mentioned earlier in this unit, many bacteria are able to sense their own population density, communicate with each other by way of secreted chemical factors, and behave as a population rather than as individual bacteria . This is referred to as cell-to-cell signaling or quorum sensing and plays an important role in pathogenicity and survival for many bacteria.

Quorum sensing involves the production, release, and community-wide sensing of molecules called autoinducers that modulate gene expression in response to the density of a bacterial population. When autoinducers produced by one bacterium cross the membrane of another, they bind to receptors in the cytoplasm. This autoinducer/receptor complex is then able to bind to DNA promoters and activate the transcription of quorum sensing-controlled genes. In this way, individual bacteria within a group are able to benefit from the activity of the entire group.

The outcomes of bacteria-host interaction are often related to bacterial population density. Bacterial virulence, that is its ability to cause disease, is largely based on the bacterium's ability to produce gene products called virulence factors that enable that bacterium to colonize the host, resist body defenses, and harm the body. If a relatively small number of a specific bacteria were to enter the body and immediately start producing their virulence factors, chances are the body's immune systems would have sufficient time to recognize and counter those virulence factors and remove the bacteria before there was sufficient quantity to cause harm. Many bacteria are able to delay production of those virulence factors by not expressing the genes for those factors until there is a sufficiently large enough population of that bacterium (a quorum). As the bacteria geometrically increase in number, so does the amount of their secreted autoinducers.

When a critical level of autoinducer is reached, the entire population of bacteria is able to simultaneously activate the transcription of their quorum-sensing genes and the body's immune systems are much less likely to have enough time to counter those virulence factors before harm is done.

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Clostridium perfringens*
- *Streptococcus pyogenes*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Clostridium difficile*
- *Streptococcus pneumoniae*

TPS Questions

Concept Map for Type II Toxins (Toxins that Damage Cell Membranes)

Self Quiz for the Ability to Produce Harmful Exotoxins: Type II Toxins

Quiz Group

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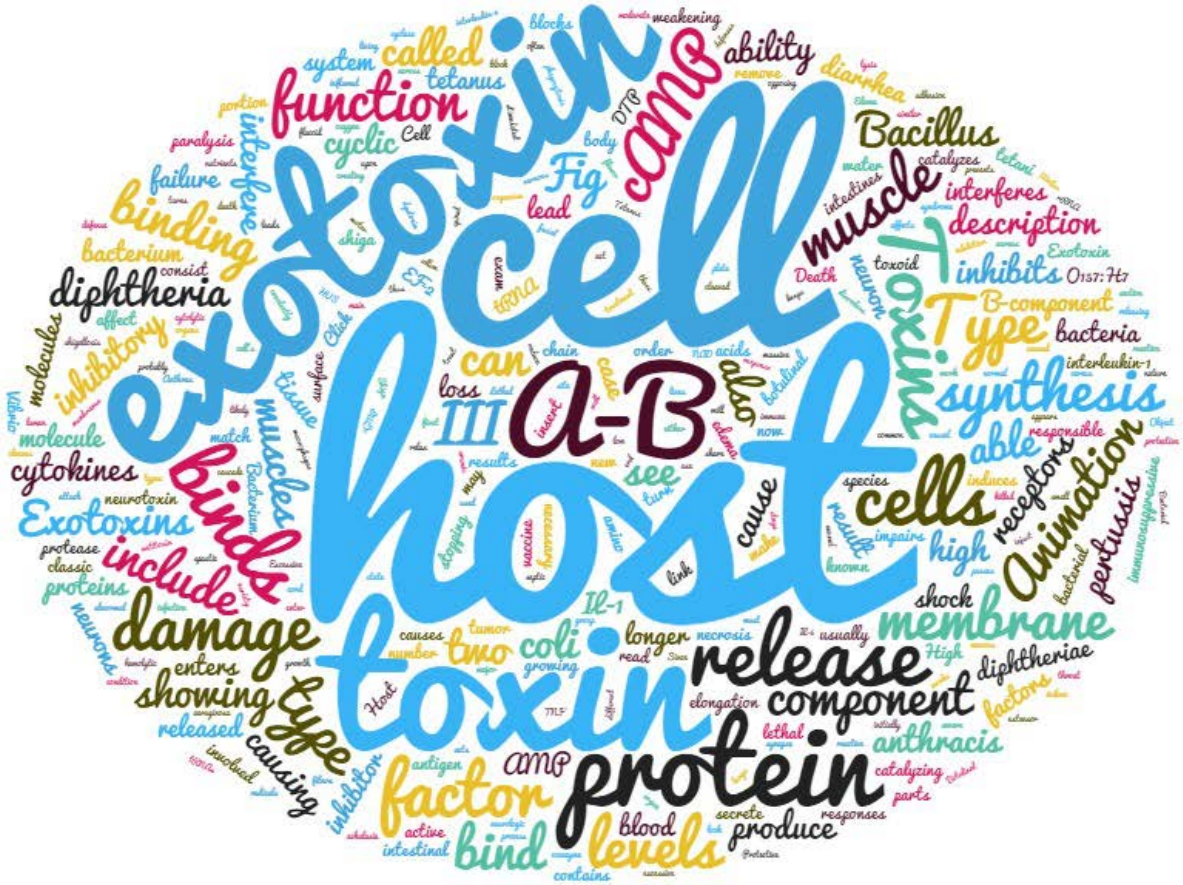
Type-III bacterial toxins
VIRULENCE FACTORS THAT DAMAGE THE HOST

C. VIRULENCE FACTORS THAT DAMAGE THE HOST

2. The Ability to Produce Harmful Exotoxins

c. Type III toxins (A-B Toxins and Other Toxins that Interfere with Host Cell Function)



Fundamental Statements for this Softchalk Lesson:

1. The classic type III toxins are A-B toxins that consist of two parts: an "A" or active component that enzymatically inactivates some host cell protein or signaling pathway to interfere with a host cell function; and a "B" or binding component that binds the exotoxin to a receptor molecule on the surface of the host cell membrane and determines the type of host cell to which the toxin is able to affect.
2. Examples include the diphtheria exotoxin produced by Corynebacterium diphtheria, the cholera exotoxin produced by Vibrio cholerae, certain enterotoxins that cause loss of electrolytes and water resulting in diarrhea, the pertussis exotoxin produced by Bordetella pertussis, shiga toxin, produced by species of Shigella and enterohemorrhagic Escherichia coli (EHEC), the anthrax toxins produced by Bacillus anthracis, the tetanus exotoxin of Clostridium tetani, and the botulism exotoxin of Clostridium botulinum.

Common Course Objectives

1. Recall the factors that influence disease severity.

2. Explain how diseases can be transmitted.

3. Describe virulence factors that may harm the host and give relevant examples.

4. Determine the difference between endotoxin and exotoxin.

5. Discuss the significance of exotoxins and recognize specific examples.

6. Discuss how exoenzymes aid in the development of disease.

Detailed Learning Objectives

1.* Define A-B toxins and state the functions of the A component and the B component.

2. State how the following exotoxins cause harm and name a bacterium producing each:

a. diphtheria exotoxin

b. cholera exotoxin

c. enterotoxins

d. shiga toxin

e. anthrax lethal toxin and edema toxin

f. botulism exotoxin

g. tetanus exotoxin

(*) = Common theme throughout the course

Highlighted Bacterium

1. Read the description of *Corynebacterium diphtheriae* and match the bacterium with the description of the organism and the infection it causes.

2. Read the description of *Bacillus anthracis* and match the bacterium with the description of the organism and the infection it causes.

TPS Questions

In this section on Bacterial Pathogenesis we are looking at virulence factors that damage the host. Virulence factors that damage the host include:

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response.

2. The ability to produce harmful exotoxins.

3. The ability to induce autoimmune responses.

We will now look at the ability of bacteria to produce type III exotoxins.

2. The Ability to Produce Harmful Exotoxins

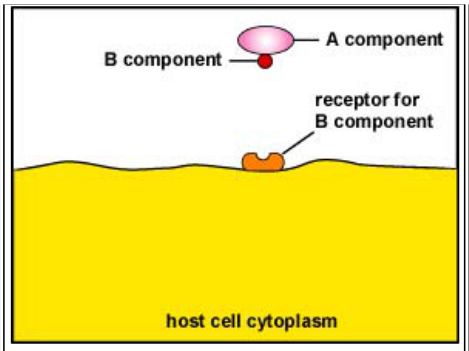
c. Type III toxins (A-B Toxins and Other Toxins that Interfere with Host Cell Function)

The classic type III toxins are A-B toxins that consist of two parts **(see Fig. 1)**:

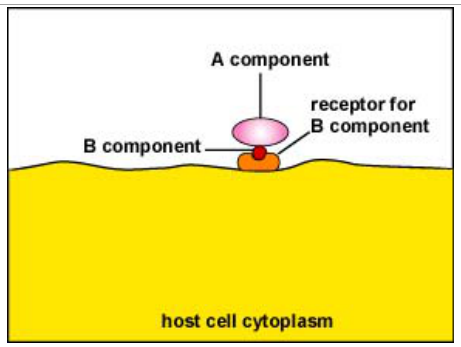
1. An **"A" or active component that enzymatically inactivates some host cell intracellular target or signaling pathway to interfere with a host cell function**; and

2. A "**B" or binding component (see Fig. 2)** that **binds the exotoxin to a receptor molecule on the surface of the host cell membrane** and determines the type of host cell to which the toxin is able to affect.

Fig. 1: A-B Toxins	Fig. 2: Binding of A-B toxins



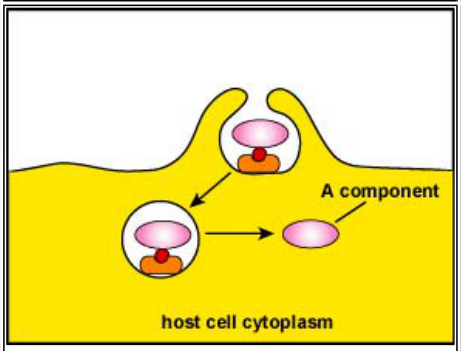
A-B toxins consist of two parts, an A (active) component and a B (binding) component.



The B (binding) component of the exotoxin binds to a receptor on the surface of a susceptible host cell.

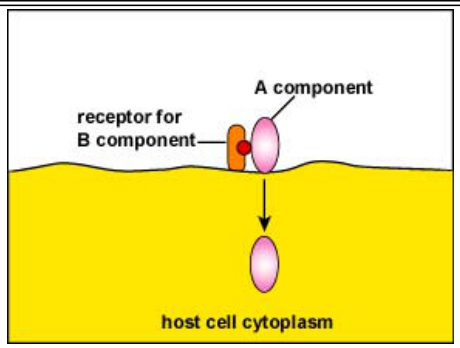
Once the exotoxin binds, it is translocated across the host cell membrane. Some A-B toxins enter by endocytosis **(see Fig. 3)**, after which the A-component of the toxin separates from the B-component and enters the host cell's cytoplasm. Other A-B toxins bind to the host cell and the A component subsequently passes directly through the host cell's membrane and enters the cytoplasm **(see Fig. 4)**.

Fig. 3: Entry of A-B Toxins by Endocytosis



This A-B toxin enters the host cell by endocytosis and subsequently causes harm by the ADP-ribosylation of a target host cell protein.

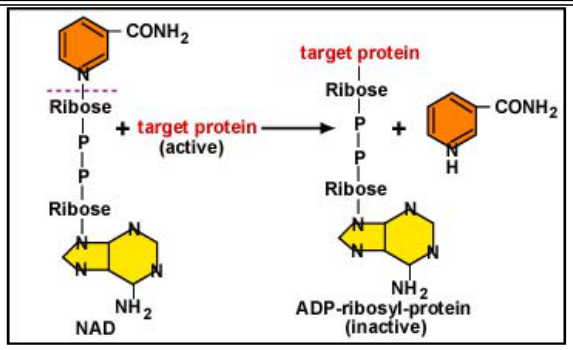
Fig. 4: Entry of A Component of A-B Toxins by Direct Passage through the Cytoplasmic Membrane of the Host Cell



After binding to the host cell receptor, the A component of this A-B toxin enters the host cell by directly passing through the cytoplasmic membrane of the host cell. It subsequently causes harm by the ADP-ribosylation of a target host cell protein.

The A components of most A-B toxins then catalyze a reaction by which they **remove the ADP-ribosyl group from the coenzyme NAD and covalently attach it to some host cell protein**, a process called ADP- ribosylation **(see Fig. 5)**. This **interferes with the normal function of that particular host cell protein** that, in turn, determines the type of damage that is caused. Some A-B toxins work differently.

Fig. 5: ADP- Ribosylation of a Target Host Protein by an A-B Toxin



The A component of most A-B toxins catalyzes ADP-ribosylation of host cell target proteins. The ADP-ribosyl group is removed from the coenzyme NAD (see dashed line) and is covalently attached to a host cell target protein. This causes the inactivation of that target protein.

Animation of an A-B toxin binding to and penetrating a susceptible host cell.

The body's major **defense against exotoxins** is the production of **antitoxin antibodies**. Once the antibody binds to the exotoxin, the toxin can no longer bind to the receptors on the host cell membrane.

Flash animation showing the neutralization of exotoxins with antibodies.

html5 version of animation for iPad showing the neutralization of exotoxins with antibodies.

The Fab portion of the antibodies made against epitopes of the binding site of an exotoxin blocks the exotoxin from binding to the host cell membrane. As a result, the toxin can not enter the cell and cause harm.

Examples of A-B toxins include:

1. **Diphtheria exotoxin**, produced by *Corynebacterium diphtheriae*. This toxin **interferes with host cell protein synthesis** by catalyzing the ADP-ribosylation of host cell elongation factor 2 (EF-2), necessary in order for tRNA to insert new amino acids into the growing protein chain. This results in cell death. Initially cells of the throat are killed by the toxin. The toxin is also released into the blood where it damages internal organs and can lead to organ failure. The "D" portion of the DTP vaccine contains **diphtheria toxoid** to stimulate the body to make neutralizing antibodies against the binding component of the diphtheria exotoxin. Once the antibody binds to the exotoxin, the toxin can no longer bind to the receptors on the host cell membrane.

2**. Cholera exotoxin** (choleragen), produced by *Vibrio cholerae*. This exotoxin catalyzes the ADP-ribosylation of a host cell protein called Gs that turns the synthesis of a metabolic regulator molecule called cyclic AMP (cAMP) on and off. In this case, synthesis stays turned on. High levels of cAMP **block intestinal epithelial cells from taking in sodium from the lumen of the intestines and stimulates them to secrete large quantities of chloride. Water and other electrolytes osmotically follow**. This causes **loss of fluids, diarrhea, and severe dehydration**.

Movie showing the effect of cholera exotoxin on human cells.

The Theriot Lab Website at Stanford University Medical School.

3. **Enterotoxins**. A number of bacteria produce exotoxins that bind to the cells of the small intestines. Most of these toxins catalyze the ADP-ribosylation of host cell proteins that turn the synthesis of the metabolic regulator molecules cyclic AMP (cAMP) or cyclic GMP on and off in intestinal mucosal cells. High levels of cAMP and cGMP **cause loss of electrolytes and water that results in diarrhea.** Organisms producing enterotoxins include *Clostridium perfringens*,and *Bacillus cereus*. (As mentioned under Type I toxins, the enterotoxins of *Staphylococcus aureus* and enterotoxogenic *E. coli* work differently, functioning as superantigens.)

4. **Pertussis exotoxin**, produced by *Bordetella pertussis*. The pertussis exotoxin catalyzes the ADP-ribosylation of a host cell protein called Gi leading to high intracellular levels of cAMP. This disrupts cellular function. In the respiratory epithelium, the high levels of cAMP results in increased respiratory secretions and mucous production and contribute to coughing. In the case of phagocytes, excessive cAMP decreases phagocytic activities such as chemotaxis, engulfment, killing. In the blood, the toxin results in increased sensitivity to histamine. This can result in increased capillary permeability, hypotension and shock. It may also act on neurons resulting in encephalopathy.

5. *Pseudomonas aeruginosa* produces a variety of toxins that lead to tissue damage in the host. Type II toxins include:

a. Exotoxin A: **interferes with host cell protein synthesis** by catalyzing the ADP-ribosylation of host cell elongation factor 2 (EF-2), necessary in order for tRNA to insert new amino acids into the growing protein chain; is also immunosuppressive.

b. Exotoxin S: inhibits host cell protein synthesis causing tissue damage; is immunosuppressive.

6. **Shiga toxin**, produced by species of *Shigella* and enterohemorrhagic *Escherichia coli* (EHEC) such as such as *E. coli* O157:H7. This toxin is an A-B toxin that **cleaves host cell rRNA** and prevents the attachment of charged tRNAs thus stopping host cell protein synthesis. The shiga toxin also enhances the LPS-mediated release of cytokines such as Il-1 and TNF-alpha and appears to be responsible for a complication of shigellosis and *E. coli* O157:H7 infection called **hemolytic uremic syndrome** (HUS), probably by causing **blood vessel damage**.

For more information: Hemolytic uremic syndrome (HUS)

7. **Anthrax toxins**, produced by *Bacillus anthracis.* In the case of the two anthrax exotoxins, two different A-components known as **lethal factor (LF)** and **edema factor (EF)** share a common B-component known as **protective antigen (PA)**. Protective antigen, the B-component, first binds to receptors on host cells and is cleaved by a protease creating a binding site for either lethal factor or edema factor.

a. **Lethal factor** is a protease that inhibits mitogen-activated kinase-kinase. At **low levels, LF inhibits the release of proinflammatory cytokines such as**

interleukin-1 (IL-1), tumor necrosis factor-alpha, (TNF-alpha), and NO. This may initially **reduce immune responses** against the organism and its toxins. But **at high levels, LF is cytolytic for macrophages, causing release of high levels of interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and NO**. Excessive release of these cytokines can lead to a **massive inflammatory response and the shock cascade**, similar to septic shock.

b. **Edema factor** is an adenylate cyclase that **generates cyclic AMP in host cells. It impairs phagocytosis, and inhibits production of TNF and interleukin-6 (IL-6) by monocytes**. This most likely impairs host defenses.

Highlighted Bacterium: *Corynebacterium diphtheriae*
Click on this link, read the description of *Corynebacterium diphtheriae*, and be able to match the bacterium with its description on an exam.

Highlighted Bacterium: *Bacillus anthracis*
Click on this link, read the description of *Bacillus anthracis*, and be able to match the bacterium with its description on an exam.

There are a number of other bacterial exotoxins that cause damage by interfering with host cell function. They include the following:

1. **Botulinal exotoxin**, produced by *Clostridium botulinum*. This is a neurotoxin that acts peripherally on the autonomic nervous system. For muscle stimulation, acetylcholine must be released from the neural motor end plate of the neuron at the synapse between the neuron and the muscle to be stimulated. The acetylcholine then induces contraction of the muscle fibers. The botulism exotoxin binds to and enters the presynaptic neuron and blocks its release of acetylcholine. This causes a **flaccid paralysis**, a weakening of the involved muscles. Death is usually from respiratory failure. While two exotoxins of *C. botulinum* catalyze ADP-ribosylation of host cell proteins, the botulinal toxin that affects neurons does not. Since the botulinal toxin is able to cause a weakening of muscles, it is now being used therapeutically to treat certain neurologic disorders such as dystonia and achalasia that result in abnormal sustained muscle contractions, as well as a treatment to remove facial lines.

Animation showing acetylcholine-induced contraction of a muscle.
Animation showing botulism exotoxin blocking acetylcholine release.

2. **Tetanus exotoxin** (tetanospasmin), produced by *Clostridium tetani*. This is a neurotoxin that binds to inhibitory interneurons of the spinal cord and blocks their release of inhibitor molecules. It is these inhibitor molecules from the inhibitory interneurons that eventually allow contracted muscles to relax by stopping excitatory neurons from releasing the acetylcholine that is responsible for muscle contraction. The toxin, by blocking the release of inhibitors, keeps the involved muscles in a state of contraction and leads to **spastic paralysis**, a condition where opposing flexor and extensor muscles simultaneously contract. Death is usually from respiratory failure. The "T" portion of the DTP vaccine contains **tetanus toxoid** to stimulate the body to make neutralizing antibodies against the binding component of the diphtheria exotoxin. Once the antibody binds to the exotoxin, the toxin can no longer bind to the receptors on the host cell membrane.

Animation showing inhibition of muscle contraction by an inhibitory interneuron.
Animation showing tetanus exotoxin blocking inhibitor release from an inhibitory interneuron.

3. Neutrophil activating protein, produced by *Helicobacter pylori*. Neutrophil activating protein promotes the adhesion of human neutrophils to endothelial cells and the production of reactive oxygen radicals. The toxin induces a moderate inflammation that promote *H. pylori* growth by the release of nutrients factors from the inflamed tissue.

Flash animation showing the induction of stomach ulcers by *Helicobacter pylori*.
Helicobacter pylori, by means of its flagella, is able to swim through the mucus layer of the stomach or intestines and adhere to the epithelial cells of the mucous membranes. Here the pH is near neutral. To also help protect the bacterium from the acid, *H. pylori* produces an acid-inhibitory protein that blocks acid secretion by surrounding parietal cells in the stomach. The bacterium then releases toxins that lead to excessive production of cytokines and chemokines, as well as mucinase and phospholipase that damage the gastric or intestinal mucosa. The cytokines and chemokines, in turn, result in a massive inflammatory response. Neutrophils leave the capillaries, accumulate at the area of infection, and discharge their lysosomes for extracellular killing. This not only kills the bacteria, it also destroys the mucus-secreting mucous membranes of the stomach. Without this protective layer, gastric acid causes ulceration of the stomach or intestines. (Note that *H. pylori* is actually a spiral-shaped bacteria with a lophotrichous arrangement of flagella but showing this in the animation is beyond my technical abilities.)

TPS Questions

Concept Map for Type III Toxins (A-B Toxins and Other Toxins that Interfere with Host Cell Function)

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Corynebacterium diphtheriae*
- *Vibrio cholerae*
- *Clostridium perfringens*
- *Bacillus cereus*
- *Staphylococcus aureus*
- *Bordetella pertussis*
- *Pseudomonas aeruginosa*
- *Shigella* species
- *Clostridium botulinum*
- *Clostridium tetani*
- *Helicobacter pylori*

Self Quiz for Type III toxins (A-B Toxins and Other Toxins that Interfere with Host Cell Function)

Quiz Group

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The ability of bacteria to induce autoimmune responses
VIRULENCE FACTORS THAT DAMAGE THE HOST

C. VIRULENCE FACTORS THAT DAMAGE THE HOST

3. The Ability to Induce Autoimmune Responses

Fundamental Statements for this Softchalk Lesson:

1. Autoimmunity is when the body's immune defenses mistakenly attack the body and sometimes certain bacteria can serve as a trigger for this response.
2. One way bacteria can trigger autoimmunity by stimulating the production of cross-reacting antibodies. These are antibodies made in response to bacterial antigens then accidentally cross-react with and destroy host cells to which they have bound. An example is rheumatic fever following Streptococcus pyogenes infection.
3. Another way autoimmunity can be triggered by certain bacteria is by stimulating the production of soluble antigen-antibody (immune) complexes. These immune complexes can lodge in filtering units such as the kidneys where they activate the complement pathway and trigger an inflammatory response then destroys kidney tissues. An example of this is acute glomerulonephritis that sometimes following infection by Streptococcus pyogenes.

Common Course Objectives

1. Recall the factors that influence disease severity.
2. Explain how diseases can be transmitted.
3. Describe virulence factors that may harm the host and give relevant examples.
4. Briefly describe the mechanism behind the different types of hypersensitivity and give an example of each.

Detailed Learning Objectives

1. State what is meant by autoimmunity.
2. Name 3 bacterial diseases that may result from autoimmunity.

TPS Questions

In this section on Bacterial Pathogenesis we are looking at virulence factors that damage the host. Virulence factors that damage the host include:

1. The Ability of PAMPs to Trigger the Production of Inflammatory Cytokines that Result in an Excessive Inflammatory Response.

2. The ability to produce harmful exotoxins.

3. The ability to induce autoimmune responses.

We will now look at the ability of bacteria to induce autoimmunity.

3. The Ability to Induce Autoimmune Responses

Autoimmunity is when **the body's immune defenses mistakenly attack the body** and sometimes certain bacteria can serve as a trigger for this response.

One way bacteria can do this is by **inducing the production of cross-reacting antibodies** and possibly **auto-reactive cytotoxic T-lymphocytes or CTLs**. These are **antibodies and CTLs made in response to bacterial antigens that accidentally cross react with epitopes on host cells**. As a result, the antibodies and CTLs wind up destroying the host cells to which they have bound. Furthermore, when the antibodies activate the classical complement pathway, this further stimulates the **inflammatory response** resulting in more tissue damage. **Rheumatic fever** triggered by rheumatogenic strains of *Streptococcus pyogenes* is an example. Antibodies and CTLs stimulated by antigens of *S. pyogenes* cross-react with heart and joint tissues damaging the heart and joints.

Animation showing opsonization of cells during Type-II hypersensitivity.
Animation showing MAC lysis of cells during Type-II hypersensitivity.

Animation showing ADCC by NK cells.
Animation showing ADCC apoptosis by NK cells.

For more information: Preview of Type II Hypersensitivities

Another way autoimmunity can be triggered by certain bacteria is by **stimulating the production of soluble immune complexes**. When high levels of circulating antibodies react with certain bacterial antigens, they form large amounts of immune complexes (antibodies bound to antigens). These immune complexes can lodge in filtering units such as the kidneys where they **activate the complement pathway**. The resulting **inflammatory response** then destroys kidney tissues. An example of this is **acute glomerulonephritis** that sometimes following infection by *Streptococcus pyogenes*.

Animation showing inflammation and tissue death during Type-III hypersensitivity.

For more information: Preview of Type III Hypersensitivities

Two other possible examples of bacterial induced autoimmunity are **chronic Lyme disease** (arthritis, neurological abnormalities, and heart damage) following infection by *Borrelia burgdorferi*, and **tertiary syphilis** (heart damage, neurological abnormalities, and destructive skin lesion) following infection by *Treponema pallidum*.

TPS Questions

Concept Map for the Ability to Induce Autoimmune Responses

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Streptococcus pyogenes*
- *Treponema pallidum*
- *Leptospira*
- *Borrelia burgdorferi*

Autoimmunity will be discussed in greater detail under Hypersensitivies in Unit 6.

Self Quiz for the Ability to Induce Autoimmune Responses

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