6.1B: Gram-Negative Bacterial PAMPs

Skills to Develop

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 and name 3 common Gram-negative bacteria that cause HAIs.

Highlighted Bacterium

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

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 (def). 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 (def), 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 (def) and activation of the coagulation pathway (def).

Cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), and interleukin-8 (IL-8) are known as inflammatory cytokines (def) because they promote inflammation. Some cytokines, such as IL-8, are also known as chemokines (def). 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 (def) 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 (see Figure 1) is also known as endotoxin (def). 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.

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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 (def) and dendritic cells (def)** (see Figure 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 (def) TLR-4 (def) 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 Figure 2). The cytokines then bind to cytokine receptors on target cells and initiate an inflammatory response (def). They also activate both the complement pathways (def) and the coagulation pathway (def) (see Figure 2).
4. The binding of LPS molecules to their TLRs on the surfaces of phagocytic white blood cells called neutrophils (def) causes them to release proteases (def) and toxic oxygen radicals (def) for extracellular killing. Chemokines (def) such as interleukin-8 (IL-8) also stimulate extracellular killing. In addition, LPS and cytokines stimulate the synthesis of a vasodilator called nitric oxide.

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 (def), macrophages (def), and dendritic cells (def) 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 (def) and the coagulation pathway (def), and generally stimulating immune responses (see Figure 2). Also as a result of these cytokines, circulating phagocytic white blood cells such as neutrophils (def) and monocytes (def) stick to the walls of capillaries, squeeze out and enter the tissue, a process termed diapedesis (def). 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 Gram-negative PAMPs are released resulting in excessive cytokine production by the defense cells and this can harm the body (see Figure 3). In addition, neutrophils (def) 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 (def), tissue destruction, wasting, acute respiratory distress syndrome (ARDS) (def), disseminated intravascular coagulation (DIC) (def), and damage to the vascular endothelium. This can
result in shock (def), multiple system organ failure (MSOF), and often death.

Exercise: Think-Pair-Share Questions

1. Describe the mechanism by which gram-negative bacteria initiate the inflammatory response and activate the coagulation pathway and the complement pathway.
2. State how this can be both beneficial and harmful to the body.

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

1. A drop in blood volume or hypovolemia (def). 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 (def). This is a result of the following events:
   a. Prolonged vasodilation causes decreased vascular resistance within blood vessels decreases blood pressure.
   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. The inability to deliver nutrients and oxygen to body cells or hypoperfusion (def). 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 acid end products of fermentation lead to acidosis and the wrong pH 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** (def) Ischemia is a restriction in blood supply that results in damage or dysfunction of tissues or organs.
- **Multiple system organ failure (MSOF)** (def). Multiple organs begin to fail as a result of hypoperfusion.
- **Death**.

For more information: Review of SIRS and Septic Shock from Unit 3

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

Concept map for SIRS and Septic Shock.

**Septicemia** (def) 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 **health care-associated infections (HAIs)** (def).

Other examples of damage from Gram-negative PAMPs are Gram-negative **bacterial meningitis** (def) 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 (def) 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 ([def]).

Medically important Gram-negative bacteria include such classical pathogens as *Neisseria meningitidis* ([inf]), *Salmonella* ([inf]), *Neisseria gonorrhoeae* (see photomicrograph) ([inf]), and *Hemophilus influenzae* type b ([inf]).

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** ([inf]) including urinary tract infections, wound infections, pneumonia, and septicemia. These bacteria owe much of their damage to LPS.

<table>
<thead>
<tr>
<th>Highlighted Bacterium: <em>Pseudomonas aeruginosa</em></th>
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<tr>
<td>Click on this link, read the description of <em>Pseudomonas aeruginosa</em>, and be able to match the bacterium with its description on an exam.</td>
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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 **health care-associated infections (HAIs)** ([def]). 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 HAIs in the U.S. between 1990 and 1996. There are over two million nosocomial infections per year in the U.S.

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

- 32 percent of all health care-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*
Summary

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 Health care-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.

Questions

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

1. State what is meant by endotoxin and where it is normally found. (ans)

2. Define healthcare-associated infection and name 3 common Gram-negative bacteria that cause HAIs. (ans)

3. We just learned that during a severe Gram-negative infection, LPS from the gram-negative cell wall can bind to macrophages causing their release of chemokines and cytokines and this is what then may lead to the often lethal shock cascade. Why would the human body evolve a mechanism for LPS binding to macrophages if it is potentially harmful? (ans)

4. Multiple Choice (ans)

Contributors

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