6.2C: Type III Toxins: A-B Toxins and other Toxins that Interfere with Host Cell Function

Skills to Develop

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

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.

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

1. An "A" or active component that enzymatically inactivates some host cell intracellular target or signalling pathway to interfere with a host cell function; and
2. a "B" or binding component (see Figure 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.

Once the exotoxin binds, it is translocated across the host cell membrane. Some A-B toxins enter by endocytosis (see Figure 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 Figure 4).

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 Figure 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.

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.

Examples of A-B toxins include:

1. Diphtheria exotoxin, produced by *Corynebacterium diphtheriae* (inf). 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* (inf). 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. For a movie of showing the effect of cholera exotoxin on human cells, see the Theriot Lab Website at Stanford University Medical School. Click on "Vibrio cholerae colonizing human cells."

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 result in diarrhea. Organisms producing enterotoxins include Clostridium perfringens (inf), and Bacillus cereus (inf). (As mentioned under Type I toxins, the enterotoxins of Staphylococcus aureus (inf) and enterotoxogenic E. coli (inf) work differently, functioning as superantigens.)

4. Pertussis exotoxin, produced by Bordetella pertussis (inf). 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 (inf) 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.

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: 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.

For More Information: the Shock Cascade from Unit 2
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* (inf). 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.

   ![GIF animation showing acetylcholine-induced contraction of a muscle.](image1)
   ![GIF animation showing botulism exotoxin blocking acetylcholine release.](image2)

2. **Tetanus exotoxin** (tetanospasmin), produced by *Clostridium tetani* (inf). 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.

   ![GIF animation showing inhibition of muscle contraction by an inhibitory interneuron.](image3)
   ![GIF animation showing tetanus exotoxin blocking inhibitor release from an inhibitory interneuron.](image4)

3. **Neutrophil activating protein**, produced by *Helicobacter pylori* (inf). 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 induction of stomach ulcers by Helicobacter pylori.](image5)

**Exercise: Think-Pair-Share Questions**

Tetanus and diphtheria are very rare in the US because we immunize our population with the DTaP vaccine.
Components of the vaccine include tetanus toxoid and diphtheria toxoid.

Explain the adaptive immune mechanism by which this immunization confers protection.

Concept map for Type III Toxins (AB Toxins and Toxins that Interfere with 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

Summary

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 signalling 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.

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.

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.

2. Match the following descriptions with the exotoxin:

   _____ Produced by certain strains of *Escherichia coli* such as *E. coli* O157:H7. These toxins kill intestinal epithelial cells of the colon and cause bloody diarrhea. Less commonly, the toxins enter the blood and are carried to the kidneys where they damage endothelial cells of the blood vessels and cause hemolytic uremic syndrome (HUS). (ans)

   _____ Produced by a species of *Clostridium*. This is a neurotoxin that acts peripherally on the autonomic nervous system. This toxin binds to and enters the presynaptic neuron and blocks its release of acetylcholine. This causes a flaccid paralysis, a weakening of the involved muscles. (ans)

   _____ Produced by a species of *Clostridium*. This is a neurotoxin that binds to inhibitory interneurons of the spinal cord and blocks their release of inhibitor molecules. 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. (ans)

   _____ At low levels, this toxin 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, it 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. (ans)

   a. diphtheria exotoxin
   b. cholera exotoxin
   c. enterotoxins
   d. pertussis exotoxin
   e. shiga toxin
   f. anthrax lethal toxin
   g. botulism exotoxin
   h. tetanus exotoxin

3. Multiple Choice (ans)

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