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Bacterial Pathogenesis

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This lecture will cover general concepts in host-microbe interactions including how bacteria spread, how they can colonize and/or invade host cells, how they are able to evade host defenses, and how they cause disease.

But first, definitions of a few key terms:

Pathogen: A microorganism capable of producing pathology (disease) in a percentage of normal, healthy non-immune individuals. Disease is when damage or an altered physiologic state has occurred in the host. A pathogen will almost always cause disease in a non-immune host if it is administered in a sufficient dose. However, at lower doses, infection may result without overt disease (sub-clinical infection). Persons harboring a pathogen without apparent disease are referred to as **carriers**. Even though carriers do not experience symptoms of the disease, they are still capable of transmitting the disease to others (Think of 'Typhoid Mary').
Note: The goal of a pathogenic organism is to reproduce, not cause disease *per se*. Disease of the host is often associated with the propagation of the microorganism and/or the spread of the microorganism. For example, a cough facilitates transmission.

Opportunistic pathogen: Some microorganisms will not cause disease in a healthy host, will cause disease only in individuals whose normal defense mechanisms have been compromised, e.g. burn patients, organ transplant recipients who are receiving immunosuppressants, individuals with AIDS, pregnant women, and people with indwelling catheters. Opportunistic pathogens are becoming increasingly important as more immunocompromised hosts are surviving for extended time periods.

Extracellular pathogen: Bacteria or parasites that replicate outside of host cells.
Intracellular pathogen: Bacteria or parasites that replicate inside of host cells.
Whether a bacterial pathogen grows inside or outside of mammalian cells affects the type of immune defenses that it encounters.

Obligate pathogens cannot be (or have not been) found anywhere but in association with their host. (Note: This has implications for disease eradication!) **Obligate intracellular pathogens** can grow only inside of host cells, and cannot be cultured extracellularly. Thus, all viruses are obligate intracellular pathogens. Chlamydia is an example of a bacterial obligate intracellular pathogen.

Facultative pathogens can grow or survive in the environment as well as in their host. **Facultative intracellular pathogens** can grow both inside and outside of cells and can be cultured on an agar surface in the laboratory.

Virulence refers to the degree of disease that a pathogen can cause. Often this is quantified in animal models by the LD50 (Lethal dose 50) which is the number of microorganisms (or amount of a toxin) required to kill 50% of the test animals. ID50 (infectious dose 50) is the number of organisms required to produce an infection in 50% of test animals. Note: Often the most successful pathogens are NOT the most virulent. It doesn't pay to kill off your host before you're had a chance to successfully reach a new host!

Virulence Factors are components of the pathogen that contribute to its ability to cause an infection. These are often defined by mutations in specific genes that result in lower virulence (an increase in LD50). Virulence factors include the obvious (toxins, adhesions, proteins involved in immune evasion) and the not so obvious (iron acquisition, special adaptations of metabolism).

The term **invasion** can be used in two different ways. The general meaning is to enter the host's tissues and disseminate, i.e. leave the mucosal surface. But invasion can also mean to enter host cells. Thus, *S. typhimurium* can enter epithelial cells (invade them), and can cause invasive disease by disseminating to liver and spleen.

Bacterial infections can be **acute**, **chronic**, or **latent**. Acute infections are short-lived and sometimes severe. The bacteria operate with a "get in and get out" strategy and usually have moved on before adaptive immunity kicks in. A good example of a bacterium that causes an acute infection is Vibrio cholerae (causes cholera). Chronic infections persist over a long time. For example, Helicobacter pylori (causative agent of stomach ulcers and stomach cancer) can often persist for the life of the host. Latency refers to a period of inactivity. Thus, Tb can infect a person, be sequestered (and often inert) in a walled off tubercle (no symptoms), and then break out of the tubercle to cause active disease if the host experiences suppression of its immune system (e.g. second infection with another pathogen such as HIV).

How Do Bacteria Cause Disease?

"Good fences make good neighbors." As discussed in the previous lecture, bacteria cover almost all the surfaces of us that are in contact with the outside environment and this colonization by our natural microbiota is largely beneficial to us. Importantly, the body has barriers that keep bacteria from reaching sites that need to remain sterile. It is usually when bacteria breach these barriers that disease ensues.

Today we'll discuss three main ways in which bacteria (or bacterial products) breach the body's barriers and cause disease: **intracellular growth of bacteria**, disruption of host cell function from the outside (**extracellular pathogens**), and **extracellular toxins** that kill or alter host cells.

Slide #1

To be successful, all pathogens have to be able to:

-Gain Entry
-Establish a niche in which to replicate (this can be at the site of entry, i.e. on a mucosal surface, or in deeper tissue that the bacteria disseminate to after entry)
-Reach a new host

And pathogens have to accomplish all of the above in the face of the host immune system.

Slide #2

Routes of Entry/Transmission
Bacterial pathogens can be spread by all the usual routes:
Air-borne, water or food-borne, vector-borne, via sexual contact, or via a wound or cut. Many infections occur upon mucosal surfaces such as the upper respiratory tract, lower intestinal tract, and genitourinary tract. These surfaces are lined with normal flora, which on the one hand, are protective since they block access to incoming pathogens. On the other hand, the normal flora can also be a source of pathogens. Finally, because some bacteria are able to cross the placental barrier, bacteria can also be passed from mother to child.

Each route of entry has its own defense mechanisms that bacteria must be able to circumvent. For example, mucosal membranes are protected by a thick layer of mucus. Secretory IgA molecules in the mucus bind to the bacterial surface and aid phagocytes in engulfing and clearing the bacteria. In addition, mucus contains proteins that digest bacterial cell walls (lysozyme), that sequester iron (lactoferrin) and that poke holes in bacterial membranes (defensins). Bacteria that colonize mucosal surfaces have to be able to evade these defenses. Bacteria that colonize the small intestine must first make it

through the acidic pH of the stomach and then must be able to stick to a host cell surface in order to avoid being washed away.

Slides #3-9

1. Intracellular Bacterial Pathogens

Intracellular bacterial pathogens actually replicate inside mammalian cells. (Please see slide for examples of intracellular bacterial pathogens and the diseases they cause.) By definition then, this is a violation of the host/bacteria barrier. Why might bacteria prefer to grow inside cells? Intracellular growth offers access to nutrients, allows bacteria to avoid extracellular defenses of the host, and in some cases, allows bacteria to cross a barrier in order to reach deeper tissues.

Paradigm for intracellular growth: *Listeria monocytogenes*

Listeria is a Gram-positive, rod-shaped bacterium and is a food-borne pathogen. Like Salmonella, Listeria invades the epithelial cells lining the small intestine. But unlike Salmonella, Listeria uses a form of receptor-mediated entry. Listeria entry is restricted to the tips of the intestinal villi because this is where Listeria's receptor (host protein E-cadherin) is transiently exposed. Once inside the epithelial cells, Listeria is able to escape from the vacuole and divide in the host cell cytosol. There it does something quite remarkable. It polymerizes host actin at one end of the bacterium using a polarized bacterial surface protein (ActA). Polymerization of host actin at one end of the bacterium rockets the bacterium throughout the host cytosol. This movement results in the formation of pseudopods which push into neighboring cells. Each pseudopod contains a bacterium at its tip. The pseudopods are then taken up by an adjacent epithelial cell, successfully transferring the bacterium to this adjacent cell. In this way, Listeria moves from one host cell to another without ever exposing itself to the extracellular defenses of the host. Consequently, complement and antibody play little to no role in combating a Listerial infection. Instead, cytotoxic T cells are key to resolving the infection.

Slides #10-11

Listeria enters at the tips of the villi of the small intestine and then migrates down the sides of the villi by spreading from cell to cell. At the same time (but on a slower scale), epithelial cells of the villi are born down in the crypts and migrate up to the villi tips where they die and are sloughed off into the lumen of the intestine (epithelial escalator). Some people are carriers of Listeria (~1-10% of the population). Hypothesis: In carriers, Listeria sets up a protected niche in which to replicate and be maintained (with the help of cell-cell spread) and also to be continually shed back into the environment.

Slide #12

2. Extracellular bacterial pathogens

Bacteria don't have to be intracellular to cause problems for the host. Some bacteria produce toxins that travel throughout the body, enter specific host cells, and cause the disease symptoms (see next section). Other bacteria colonize mucosal surfaces, but in doing so, damage the cells of the mucosal lining, often compromising the integrity of this barrier. Extracellular pathogens can also induce inflammation which can damage host cells. In this case, it is the host's own immune response that is contributing to the disease state.

Slides #13-15

Paradigm of an extracellular pathogen: EPEC

Enteropathogenic E. coli (EPEC) is one of several forms of E. coli capable of causing disease. EPEC is a food-borne pathogen and it causes severe diarrhea in infants and children primarily in developing countries. EPEC's virulence is due to its ability to tightly associate with and damage the intestinal wall of the small intestine. EPEC first adheres to the epithelial cells loosely, and then forms a very tight and intimate attachment. Initial attachment is mediated by a bacterial pilus (BFP, bundle-forming pilus). Then, using a type three secretion system (TTSS), the bacteria inject a bacterial protein (Tir) into the epithelial cells and this bacterial protein serves as the receptor for the very tight attachment of the bacteria to the epithelial cell. An adhesion protein (intimin) on the surface of the bacteria binds to the translocated bacterial Tir protein (now in the host cell membrane) and a tight attachment is formed. Additional injected bacterial effectors (plus Tir) are responsible for interacting with the host cell cytoskeleton and causing the destruction of the microvilla and the creation of the characteristic pedestals under the attached bacteria (attaching and effacing lesions). These translocated bacterial effector proteins also disrupt the tight junctions of the epithelial lining causing a disruption of the sodium gradient and a flow of water into the intestinal lumen. End result: diarrhea.

Note: These bacterial functions are all encoded on a pathogenicity island on the EPEC chromosome. Non-EPEC strains, such as the well-behaved E. coli that is a member of our normal intestinal microbiota, lack this locus.

Slide #16-19

Bacterial evasion of extracellular host defenses:
Many extracellular pathogens live on a mucosal membrane and therefore have to contend with extracellular defenses of the host such as antibody. Here are some of the strategies used by extracellular bacteria to out-maneuver the body's antibody response.

Avoid phagocytosis by opsonizing antibodies:

IgA protease-- The most abundant immunoglobulin on the mucosal surface is secretory IgA. Many bacterial pathogens that colonize a mucosal surface produce a protease which specifically cleaves human IgA. In each case, the IgA is cleaved at the hinge region separating the Fc portion from the Fab fragment. This leaves only the Fab fragments to bind to antigens on the bacterial surface. Because it is the Fc portion of the antibody that binds to phagocytes (and also to complement), bacteria decorated with only Fab fragments do not signal the immune system and these bacteria are not phagocytosed.

Protein A-- Another way by which bacteria prevent opsonization by antibodies is to actively bind antibody to their surface, but in the **wrong** orientation. For example, *S. aureus* has a surface protein called Protein A that is able to bind the Fc portion of IgG. Just as with IgA, the Fc portion of IgG is what binds to receptors on the surface of phagocytes. Because *S. aureus* can bind IgG with the Fab portion (and not the Fc portion) facing out, the bacteria are not recognized by phagocytic cells. In addition, it is possible that this decoration may even double as a disguise and allow the bacteria to masquerade as B cells.

Decoration with Sialic acid:

Some bacteria obtain sialic acid from their host and put it onto their own cell surface. Sialic acid is minimally antigenic and serves an effective disguise for these bacteria.

Antigenic Variation (*N. gonorrhoeae* as an example):

Gonorrhea is a sexually transmitted disease that accounts for about 400,00 reported infections per year in the U.S. The infectious agent is *Neisseria gonorrhoeae*. This gram-negative coccus causes recurrent disease with little or no effective immunity from previous infection even though antibody is generated. How can the gonococcus colonize so efficiently yet evade the effects of antibody? Answer: Important surface molecules display antigenic variation!

Initial binding of the gonococcus is mediated by pili. The major pilin protein, PilE, is encoded by the *pilE* gene. This gene has 6 variable regions which are also present in many silent loci (*pilS*) elsewhere on the chromosome. Homologous recombination between the expressed *pilE* gene and the silent *pilS* loci creates antigenic variation in the pilin protein on the surface of the bacterium. New variants of the pilin protein are continuously being produced; these new variants are usually **not** recognized by host antibodies generated against the original form of the pilin protein. In this way, *N. gonorrhoeae* ensures that at least some subset of its population will continue to be able to colonize (as well as re-infect) its host.

Slides #20-22

3. Diseases primarily caused by secreted bacterial toxins

Some bacteria produce powerful toxins that are responsible for all (or almost all) the symptoms of a given disease. In these cases, the bacteria do not need to colonize the host to cause disease--the addition of toxin alone does the trick. Best example is botulism.

Botulism

The paralysis that occurs with botulism is the result of a neurotoxin produced by the spore-forming, Gram-positive bacterium *Clostridium botulinum*. (Botulinum toxin blocks signaling from the nerve terminal to the muscle cell so that the nerve can no longer tell the muscle to contract. End result: flaccid paralysis.) Clostridium species are strict anaerobes. In the presence of oxygen, they form dormant, heat-resistant spores. Because *Clostridium botulinum* spores are ubiquitous in the environment, they can end up in canned foods. If the food is not heated to sufficient temperatures to kill the spores during the canning process, the spores can germinate, grow, and produce toxin in the low-oxygen environment of the sealed can once it has cooled. **Ingestion of pre-formed toxin in the canned goods causes the disease.**

Infant Botulism ("Don't feed infants honey")

Bees often pick up Clostridium spores when collecting pollen. As a result, honey can be contaminated with relatively high numbers of Clostridium spores, which we ingest when eating the honey. In the anaerobic environment of the colon, spores can germinate and the bacteria can start to grow. In adults, however, the high density of our normal microbiota prevent Clostridium from getting a foothold and from growing to high enough titers to cause disease. Thus our resident microbiota protects us from harm. In contrast, infants less than a year old are still acquiring a full microbiota and are therefore vulnerable to Clostridium colonization and disease. And that's why you don't feed babies honey!

Slides #23-25

<u>Paradigm of an A-B toxin: Diphtheria Toxin</u>

Diphtheria is another example in which the major symptoms of the disease are attributable to the action of a single toxin, in this case, Diphtheria toxin (DT). However, in this example, the disease normally does start by the colonization of the human throat with the bacteria (*Corynebacterium diphtheriae*) that produce DT. Toxin is released in the throat, enters the bloodstream, and travels to distant sites in the body. DT binds to host cells, in particular muscle and heart cells, enters the cells, and then kills them by inhibiting host cell protein synthesis. How? DT is an enzyme that ADP-ribosylates Elongation Factor 2 thereby inactivating host cell protein synthesis. DT is very potent. A single molecule can kill a host cell!

Slides #26-27

DT is a typical A-B toxin. Originally made as a single polypeptide chain, DT is cleaved into two protein chains that are tethered by a disulfide bond. The "A" portion contains the catalytic function; the "B" portion contains the host cell binding and translocation functions. How does DT get into cells and have access to EF-2? B portion binds to its receptor on the surface of susceptible host cells. (For DT, this receptor is HB-EGF.) Toxin is then internalized by receptor-mediated endocytosis. Once inside the cell, the vacuole containing the toxin acidifies. This drop in pH causes a conformational change in the toxin protein and triggers translocation of the A portion into the host cell cytosol. Upon entry into the cytosol, the disulfide bond is reduced, and the A portion is free to find its target (EF-2) in the cytosol.

What advantage might DT offer the bacteria? Not clear. However, DT is produced when the bacteria are starved for iron. It is possible that localized cell death provides iron for the bacterial cells growing in the throat.

Best way to avoid disease is prevention with a vaccine. Antibodies against inactivated toxin (toxoid) are very effective in mopping up toxin before it has a chance to enter cells and act.

Slides # 28-31

New Diseases (and re-emergence of old diseases) can occur as a consequence of how society has (and is) changing.

Example: *Legionella pneumophila* infections of lung macrophages.

Legionella are gram-negative rods that live primarily in fresh water ponds where they parasitize amoebae. Following entry by phagocytosis, the bacteria use a Type IV Secretion System to manipulate organelle trafficking in the amoeba and create a protected vacuole for the bacteria to replicate in. Specifically, bacterial manipulation of host organelle traffic prevents the bacteria-containing vacuole from acidifying and fusing with lysosomes. In addition, the Legionella-containing vacuole recruits vesicles from the endoplasmic reticulum and becomes studded with ribosomes. Recruitment of ER vesicles serves as a source of new membrane so that the vacuole can grow and accommodate larger numbers of bacteria. Eventually, the bacteria experience stress, convert to a transmissive form, and exit the host cell by lysing it.

What does all this have to do with humans? It turns out that fresh water amoeba and human alveolar macrophages have a lot in common. And if introduced into the human lung, Legionella grows quite well in our alveolar macrophages and can cause pneumonia in susceptible hosts. Contamination of cooling towers used in modern air-conditioning systems for office buildings, hospitals, and hotels now gives humans the opportunity to breathe in Legionella and/or Legionella-infected amoebae. Result: Legionnaire's Disease!

Note: Humans are **accidental hosts** for Legionella and are also evolutionary dead ends in that humans do not transmit the bacteria.