

Lecture 41: General aspects of bacterial pathogenesis

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Suggested reading: Murray, 6th Edition Chapters 7, 18

Key Words

Pathogen	Transmission
Outbreak, epidemic, pandemic	Adhesion
Normal flora	Penetration
Infection	Invasiveness/spread
Infectious disease	Extra/intracellular parasite
Compromised host	Exotoxin
Opportunistic infection	Endotoxin and non-specific immunity
Nosocomial	Specific immunity
Koch's postulates	Autoimmunity
	Bioterrorism

Overview

Pathogenicity is a multi-factorial process that depends on: 1) the immune status of the host 2) the characteristics of the bacterial species or strain (virulence factors) and 3) the number of organisms in the exposure.

A limited number of bacterial species are responsible for the majority of infectious diseases in healthy individuals. Due to the success of vaccination, antibiotics, and effective public health measures, epidemics concerning major pathogens have largely been eradicated in the developed world. Although outbreaks, particularly in the area of food-borne infection, still arise. However the rise in antibiotic resistant organisms is of major concern. The events of Sept 11 2001, and the anthrax attacks that followed over the next few months, have forced new considerations of man-made epidemics (bioterrorism).

All humans are infected with bacteria (the normal flora) living on their external surfaces (including the skin, gut and lungs). We are constantly also exposed to environmental bacteria (including air, water, soil and food). Normally due to our host defenses most of these environmental bacteria are harmless. In compromised patients, whose defenses are weakened (e.g. immunosuppression, surgery or catheterization) bacteria from the normal flora and environment often cause opportunistic infectious diseases. When this occurs in the hospital, these infectious diseases are referred to as nosocomial. Some common bacteria found in the normal flora include: *Staphylococcus aureus*, *S. epidermidis* and *Propionibacterium acnes*

(found on the skin) and *Bacteroides* and *Enterobacteriaceae* found in the intestine (the latter in much smaller numbers).

Koch's postulates

1. The organism must always be found in humans with the infectious disease but not found in healthy ones.
2. The organism must be isolated from humans with the infectious disease and grown in pure culture.
3. The organism isolated in pure culture must initiate disease when re-inoculated into susceptible animals.
4. The organism should be re-isolated from the experimentally infected animals.

Transmission

Specific bacterial species (or strains within a species) initiate infection after being transmitted by different routes to specific sites in the human body. For example, bacteria are transmitted in airborne droplets to the respiratory tract, by ingestion of food or water, or by sexual contact.

Adhesion

Bacterial infections are usually initiated by adherence of the microbe to a specific epithelial surface of the host. Otherwise the organism is removed e.g. by peristalsis and defecation (from the gut), ciliary action, coughing and sneezing (from the respiratory tract) or urination (from the urogenital tract). Adhesion involves interactions between external constituents on the bacterial cell (adhesins) and the host cell (receptors) i.e. an adhesin-receptor interaction occurs.

S. pyogenes surface constituents include F-protein (fibronectin binding protein) and lipoteichoic acid. The protein fibronectin binds to epithelial cells and F protein/lipoteichoic acid, in turn, interact with fibronectin.

E. coli has several different types of adhesins. Type 1 fimbriae bind to mannose containing receptors. Whilst P fimbriae allow binding to galactose containing glycolipids (e.g. cerebroside) and glycoproteins present on epithelial cells. They are referred to as "P" fimbriae since they were originally shown to bind to P blood group antigens on human erythrocytes.

Penetration and spread

Some bacterial pathogens reside on epithelial surfaces, e.g. *Vibrio cholerae*. Other species are able to penetrate these cells, but remain locally. Others pass into the bloodstream, or spread from there onto other systemic sites. This often occurs in the intestine, urinary tract and respiratory tract, and much less commonly through the skin. For example, *Shigella* penetrates by

activating epithelial cells of the intestine to become endocytic but do not usually spread into the bloodstream. In other cases bacteria (e.g. *Salmonella typhi*) pass through the epithelial cells into the bloodstream. *Borrelia burgdorferi* is transmitted into the bloodstream through the skin by a tick bite. Certain degradative exotoxins secreted by some bacteria (e.g. hyaluronidase or collagenase) can loosen up the connective tissue matrix increasing the ease of passage of bacteria through these sites.

Survival in the host

Many bacterial pathogens are able to resist the cytotoxic action of plasma and other body fluids involving antibody and complement (classical pathway) or complement alone (alternate pathway) or lysozyme. Killing of extra-cellular pathogens largely occurs within phagocytes after opsonization (by antibody and/or complement) and phagocytosis. Circumvention of phagocytosis by extra-cellular pathogens is thus a major survival mechanism. Capsules (found in many pathogens) protein A (*S. aureus*) and M protein (*S. pyogenes*) function in this regard.

Protein A is a surface constituent of *S. aureus* as well as a secreted product and binds to the Fc portion of immunoglobulins blocking interaction with human cell surface receptors: Bacteria, on binding antibody, activate the classical complement cascade that results in the attachment of fragments of C3. Phagocytosis occurs after binding of the opsonized bacteria to receptors for the Fc portion of IgG or C3 regions.

Peptidoglycan, like lipopolysaccharide, can activate the alternate complement cascade. *S. pyogenes* peptidoglycan is sufficiently exposed that it is able to bind complement. The M protein of group A streptococci is an anti-phagocytic surface component. M protein binds fibrinogen from plasma which blocks complement binding to the underlying peptidoglycan layer. Thus streptococci in non-immune serum are not phagocytosed.

Intra-cellular pathogens (both obligate and facultative) must be able to avoid being killed within phago-lysosomes. This can occur from by-passing or lysing these vesicles and then residing free in the cytoplasm. Alternatively they can survive in phagosomes (fusion of phagosomes with lysosomes may be inhibited or the organism may be resistant to degradative enzymes if fusion with lysosomes occurs).

Tissue injury

Bacteria cause tissue injury primarily by several distinct mechanisms involving: 1) exotoxins 2) endotoxins and non-specific immunity, 3) specific humoral and cell mediated immunity.

1) Exotoxins

Many bacteria produce proteins (exotoxins) that modify, by enzymatic action, or otherwise destroy certain cellular structures. Effects of exotoxins are usually seen acutely, since they are sufficiently potent that serious effects (e.g. death) often result (e.g. anthrax, botulism, cholera and diphtheria). If the host survives the acute infection neutralizing antibodies (anti-toxins) are often elicited that neutralize the effect of the exotoxin. Classes of exotoxins include:

(1) Toxins which act on the extra-cellular matrix of connective tissue: e.g. *Clostridium perfringens* collagenase, *Staphylococcus aureus* hyaluronidase.

(2) Toxins which have a cell binding "B" component and an active "A" enzymatic component (A-B type toxins) include:

a) ADP-ribosylating activity: cholera toxin, *E. coli* heat labile toxin, *Pseudomonas aeruginosa* toxin and diphtheria toxin:

b) Lytic activity on 28S rRNA: shiga and shiga-like (vero) toxins.

c) Affect neurotransmission: botulinum toxin and tetanus toxin.

(3) Membrane Damaging Toxins: *Staphylococcus aureus* delta toxin

Toxins which act on the extra-cellular matrix: include proteases, collagenases and hyaluronidases. For example, *Clostridium perfringens* produces a potent collagenase, whilst *Staphylococcus aureus* produces a hyaluronidase. Damage to the connective tissue matrix (by hyaluronidase and collagenase) can "loosen up" the tissue fibers allowing the organism to spread through the tissues more readily. Also to be included in this group is the exfoliatin of *Staphylococcus aureus* which causes separation of the layers within the epidermis and is the causative agent of scalded skin syndrome in the newborn.

A -B Toxins: Such toxins consist of two components. One binds to cell surfaces and the other passes into the cell membrane or cytoplasm where it acts. The classical toxins demonstrated to act in this fashion are those of cholera and diphtheria.

(i) ADP-ribosylating exotoxins

Diphtheria toxin (produced by *Corynebacterium diphtheriae*) is coded by the phage toxin gene. The toxin is synthesized as one polypeptide chain and readily nicked into two chains held together by a disulfide bond. B binds to cells and A has the enzymatic activity. A is endocytosed and from there passes into the cytosol. Diphtheria toxin ADP-ribosylates elongation factor (EF2) in ribosomes, thus inhibiting protein synthesis. *Pseudomonas* exotoxin A has a similar mode of action to diphtheria toxin.

Cholera toxin B subunits form a ring with an A subunit inserted in the center. B binds to

gangliosides on the cell surface and provides a channel through which A penetrates. After internalization "A" ADP-ribosylates a cell membrane regulator complex (using NADH as a substrate) in turn causing activation of adenylate cyclase. Activation of adenylate cyclase causes an increase in cyclic AMP production with resulting decrease in sodium chloride uptake from the lumen of the gut and active ion and water secretion with a watery diarrhea resulting. *E. coli* labile toxin has a similar mode of action.

(ii) Toxins that inhibit protein biosynthesis

Shiga toxins (chromosomally encoded) are involved in the pathogenesis of shigellosis, whilst shiga-like toxins (phage encoded) are primarily produced by entero hemorrhagic *E. coli*. They share a common mode of action. A fragment of the A subunit passes to the ribosome where it has N-glycosidase activity on the 28S rRNA; a single adenosine residue is lysed. Diarrhea results not from active ion/water secretion, but poor water absorption due to death of epithelial cells from inhibition of protein synthesis.

(iii) Neurotransmission

Botulinum toxin acts by stopping release of acetylcholine at nerve junctions affecting muscle function (under-activity, flaccid paralysis). Tetanus toxin blocks glycine release, inactivating inhibitory neurons that stop muscle activity (over activity, rigid paralysis).

Membrane Damaging Toxins: These toxins enzymatically digest the phospholipid (or protein) components of membranes or behave as detergents. In each case holes are punched in the cell membrane and the cytoplasmic contents can leach out. The phospholipase (α toxin) of *C. perfringens* is an example of a membrane-damaging toxin. It destroys blood vessels stopping the influx of inflammatory cells. This also helps create an anaerobic environment which is important in the growth of this strict anaerobe. The δ toxin of *S. aureus* is an extremely hydrophobic protein that inserts into cell membranes and is believed to have a detergent-like action.

(2) Endotoxins and non-specific immunity

Despite the advances of the antibiotic era, around 200,000 patients will develop Gram negative sepsis each year of whom around 25-40% will ultimately die of septic shock. Septic shock involves hypotension (due to tissue pooling of fluids) disseminated intra-vascular coagulation and fever and is often fatal from massive system failure. This includes lack of effective oxygenation of sensitive tissues such as the brain. There is no effective therapy to reverse the toxic activity of lipid A.

Endotoxins are toxic components of the bacterial cell envelope. The most potent endotoxin is lipopolysaccharide. However, peptidoglycan displays endotoxin-like properties. Certain peptidoglycans are poorly biodegradable and can cause chronic as well as acute tissue injury. Endotoxins are "non-specific" inciters of inflammation. For example, cells of the

immune system and elsewhere are stimulated to release cytokines (including interleukin 1 and tumor necrosis factor). Endotoxins also activate the alternate complement pathway. The production of these cytokines results in attraction of polymorphonuclear cells into affected tissues. PG and LPS and certain other cell wall components (e.g. pneumococcal teichoic acid) are also activators of the alternate complement cascade. Thus many bacteria will bind complement encouraging their uptake and killing by phagocytes in the absence of antibody. Certain complement bi-products are also chemo-attractants for neutrophils. Endotoxins are also potent B cell mitogens, polyclonal B cell activators and adjuvants (for both antibodies and cell mediated immunity); this plays a role in the development of a suitable chronic immune response in handling the microbes if they are not eliminated acutely. Toll receptors on human cells are involved in some of these inflammatory processes.

(3) Specific immunity

Continuously generated antigens released from persisting viable microbes will subsequently elicit humoral and cell mediated immunity resulting in immunopathology. This is important in chronic infections such as tuberculosis, leprosy, Lyme disease and syphilis. Certain poorly degradable antigens (e.g. pneumococcal polysaccharide and group A streptococcal cell walls) can maintain immunopathology even in the absence of persistence of live agents. Bacterial antigens can also cross-react with host tissue antigens causing development of autoimmunity. (e.g. the M protein of *S. pyogenes* cross-reacts with mammalian myosin). Thus immunopathology can persist even after the infection and microbial antigens are eliminated.

Non-specific versus specific immunity

In a "primary" infection during the acute phase "non-specific" immunity will be of utmost importance in eradicating the infection. If the organism persists (or in a re-infection at a later date) specific immunity will be of greater significance in slowing growth of the organisms or in eliminating infection.

The immune system in resistance to infection – examples:

1. Extra-cellular parasites. Antibodies cause lysis of the organism and/or their opsonization by phagocytes at which point they are rapidly killed.
2. Intra-cellular parasites are primarily killed by cell mediated immunity.
3. Exotoxins can be neutralized by antitoxins. These can be elicited using toxoid vaccines. Toxoids are antigenic but (unlike toxins) are not toxic. For example, toxoids are used for vaccination against diphtheria.
4. Certain organisms produce IgA proteases (including *H. influenzae*, *S. pneumoniae*, *N. gonorrhoeae* and *N. meningitidis*) this helps survival on external surfaces.

Bioterrorism

Bacteria generally considered as bioterrorism agents are often ones that are effective through the airborne route and to which most people are not normally exposed or vaccinated. The major ones include *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis* and *Brucella melitensis*. Other routes (e.g. food and water) are also possible. Potential bioterrorism agents are naturally transmitted from animals and are thus discussed under zoonotic infections (Dr. Karen Fox). Unlike chemical attack (minutes-hours) in an attack with live biological agents symptoms may not be seen till days later. In the earliest stages of the disease (first several days) bioterrorism manifests as coughs and colds. Physicians must be on the lookout for clusters of patients appearing from common sources. Laboratory speciation is extremely important, since these agents are rarely seen clinically. Ideally rapid microbiological biodetection (i.e. without culture) is needed (allowing effective treatment) and environmentally (to localize the source). Unfortunately, the latter technology remains to be developed.

Some Organisms of Medical Interest

Gram negative aerobic cocci

Neisseria

Spirochetes

Treponema

Borrelia

Leptospira

Spiral, Gram negative

Campylobacter,

Helicobacter

Gram negative aerobic rods

Pseudomonas

Burkholderia

Bordetella

Francisella

Gram negative facultative rods

Enterobacteriaceae

Escherichia

Salmonella

Shigella

Yersinia

Enterobacter

Proteus

Serratia

Edwardsiella

Curved Gram negative facultative rods

Vibrio

Other Gram negative bacteria

Bartonella

Brucella

Hemophilus

Legionella

Gram negative anaerobic rods

Bacteroides

Gram positive cocci (facultative anaerobes)

Streptococcus

Staphylococcus

Gram positive anaerobic cocci

Peptococcus

Peptostreptococcus

Endospore forming Gram positive rods

Bacillus (aerobic)

Clostridium (anaerobic)

Gram positive asporogenous aerobic rods

Listeria

Erysipelothrix

Actinomycetes and related organisms

Corynebacterium

Mycobacterium

Nocardia

Actinomyces

Corynebacterium-like in appearance

Propionibacterium

Simple Gram negative bacteria

Chlamydia

Mycoplasma, Ureaplasma

Rickettsia, Coxiella

Ehrlichia

SOME MAJOR EXOTOXINS

<u>Organism</u>	<u>Disease</u>	<u>Toxin</u>	<u>Further information</u>
<i>Bacillus anthracis</i>	Anthrax	Edema toxin	Edema factor/protective antigen complex Adenylate cyclase
		Lethal toxin	Lethal factor/protective antigen complex Metalloprotease
<i>Bordetella pertussis</i>	Whooping cough	Pertussis toxin Adenylate cyclase Dermonecrotic toxin	ADP ribosylation
<i>Clostridium botulinum</i>	Botulism	Botulinum toxin	Blocks release of acetylcholine
<i>Clostridium difficile</i>	Pseudo-membranous colitis	Enterotoxin	
<i>Clostridium perfringens</i>	Gas gangrene	α toxin	Phospholipase (lecithinase) Hyaluronidase
	Food poisoning	Enterotoxin	
<i>Clostridium tetani</i>	Tetanus	Tetanospasmin	Blocks inhibitory neurones
<i>Corynebacterium diphtheriae</i>	Diphtheria	Diphtheria toxin	Inhibits elongation factor 2 (EF2) by ADP-ribosylation
<i>Escherichia coli</i>	Diarrhea (ETEC)	Heat labile toxin Heat stable toxin	Activates adenyl cyclase Activates guanyl cyclase
		Hemorrhagic colitis	Vero toxin

<u>Organism</u>	<u>Disease</u>	<u>Toxin</u>	<u>Further information</u>
<i>Pseudomonas aeruginosa</i>	Diseases of compromised host	Exotoxin A	Inhibits EF2
<i>Staphylococcus aureus</i>	Opportunistic Infections	α - δ toxins leucocidin	
	Toxic shock	Toxic shock toxin	
	Food poisoning	Enterotoxin	
	Scalded skin syndrome	Exfoliatin	
<i>Streptococcus pyogenes</i>	Scarlet fever Toxic shock	Erythrogenic/ Pyrogenic Toxin	
<i>Shigella dysenteriae</i>	Bacillary dysentery	Shiga toxin,	Inhibits protein synthesis by lysing 28S rRNA
<i>Vibrio cholerae</i>	Cholera	Cholera toxin,	Activates membrane adenyl cyclase by ADP-ribosylation