Lecture-21
Infection and Disease

Topics:
Definitions
Generalized Stages of Infection
Virulence factors
Toxins
Normal Flora

Definitions

- **infection**
  - growth and multiplication of pathogen on or within host

- **infectious disease**
  - disease resulting from infection

- **pathogen**
  - any microorganism organism that causes infectious disease
  - **primary pathogen** – causes disease by direct interaction with host
  - **opportunistic pathogen** – causes disease only under certain circumstances

Factors impacting outcome of host-pathogen relationships

- **number of organisms present**

- **virulence of pathogen**
  - virulence factors
    - products or structural components that contribute to virulence or pathogenicity

- **host’s defenses or degree of resistance**
Pathogenesis of Bacterial Diseases

- maintain a reservoir
  - place to live before and after causing infection
- be transported to host
- adhere to, colonize, and/or invade host
- multiply or complete life cycles on or in host and initially evade host defenses
- damage host
- leave host and return to reservoir or enter new host

Reservoir of the Bacterial Pathogen

- for human pathogens, most common reservoirs are:
  - other humans
  - animals
  - environment

Transport of the Bacterial Pathogen to the Host

- direct contact
  - e.g., coughing, sneezing, body contact
- indirect contact
  - vehicles (e.g., soil, water, food)
  - vectors – living organisms that transmit pathogen
  - fomites – inanimate objects that harbor and transmit pathogens

Pathogenesis of Bacterial Diseases
Attachment and Colonization by the Bacterial Pathogen

• adherence
  – mediated by special molecules or structures called adhesins

• colonization
  – establishment of a site of microbial reproduction on or within host

Mechanisms of Adherence to Cell or Tissue Surfaces

• Specific adherence mostly involves permanent formation of many specific lock-and-key bonds between complementary molecules on each cell surface.

- Nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eukaryotic cell surface.
  1. hydrophobic interactions
  2. electrostatic attractions
  3. Brownian movement
  4. recruitment and trapping by biofilm polymers

Mechanisms of Adherence to Cell or Tissue Surfaces

Specific adherence factors used by pathogens to facilitate attachment

<table>
<thead>
<tr>
<th>Factor</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocalyx</td>
<td>Pathogenic Escherichia coli—glycocalyx promotes adherence to the basal border of intestinal villi.</td>
</tr>
<tr>
<td>Adherence proteins</td>
<td>Shigella flexneri—fimbriae, and Salmonella enterica—pili bind to complementary molecules on the eukaryotic cell surface.</td>
</tr>
<tr>
<td>Lipoteichoic acid</td>
<td>Nontypeable H. influenzae—lipoteichoic acid facilitates adherence to respiratory mucosa.</td>
</tr>
<tr>
<td>Fimbriae (pili)</td>
<td>Neisseria gonorrhoeae—pili facilitate adherence to uroepithelial surfaces and bind to carbohydrate antigens on uroepithelial cells.</td>
</tr>
</tbody>
</table>

Figs. 21.13 & 21.14
Experiments

• Several types of experiments provide **direct evidence that receptor and/or adhesin molecules mediate specificity** of adherence of bacteria to host cells or tissues.

1. The bacteria will bind isolated receptors or receptor analogs.

2. The isolated adhesins or adhesin analogs will bind to the eukaryotic cell surface.

3. Adhesion of the bacterium to the eukaryotic cell surface is inhibited by:
   a. isolated adhesin or receptor molecules
   b. adhesin or receptor analogs
   c. enzymes and chemicals that specifically destroy adhesins or receptors
   d. antibodies specific to surface components (i.e., adhesins or receptors)

Invasion of the Bacterial Pathogen

• can be active penetration of host’s mucous membranes or epithelium

• can be passive penetration
  – e.g., skin lesions, insect bites, wounds

• once below mucous membrane, bacterium can spread to deeper tissues
  – involves production of specific products and/or enzymes that promote spreading

Definitions

• Localized infection vs. systemic infection
  – **Localized Infection**
    • An infection that is restricted to a specific location or region within the body of the host
  – **Systemic Infection**
    • An infection that has spread to several regions or areas in the body of the host

INVASION

• **Spreading Factors** is a descriptive term for a family of bacterial enzymes that affect the physical properties of tissue matrices and intercellular spaces, thereby promoting the spread of the pathogen.

• **Collagenase** is produced by *Clostridium*. It breaks down collagen, the framework of muscles, which facilitates gas gangrene due to these organisms.

• **Neuraminidase** is produced by intestinal pathogens such as *Vibrio cholerae* and *Shigella dysenteriae*. It degrades neuraminic acid (also called sialic acid), an intercellular cement of the epithelial cells of the intestinal mucosa.

• **Hemolysins**, notably produced by staphylococci (i.e., alpha toxin), streptococci (i.e., streptolysin) and various clostridia, may be channel-forming proteins or phospholipases that destroy red blood cells and other cells by lysis.
Growth and Multiplication of the Bacterial Pathogen

- occurs when pathogen finds appropriate environment within host
- Temperature, pH, oxygen, nutrients
  - Soluble nutrients are in limited supply (sugars, amino acids, organic acids)
  - Vitamins and growth factors are not always unavailable
  - Trace elements (e.g., Fe) may also be in short supply
  - Host produces transferrin proteins that bind Fe, keeping it away from pathogens.
  - Some pathogens produce siderophores which can remove the Fe from the transferrin
- some bacteria invade specific cells
- some actively growth in blood plasma
  - bacteremia – presence of viable bacteria in blood
  - septicemia – presence of bacteria or their toxins in bloodstream

Regulation of Bacterial Virulence Factors

- often environmental factors control expression of virulence genes
  - e.g., Corynebacterium diphtheriae
    - gene for diphtheria toxin regulated by iron
  - e.g., Bordetella pertussis
    - expression of virulence genes increased at body temperature
  - e.g., Vibrio cholerae
    - gene for cholera toxin regulated by pH, temperature and other factors

Pathogenicity Islands

- large segments of DNA that carry virulence genes
- acquired during evolution of pathogen by horizontal gene transfer
Definitions

• **Pathogenicity and Virulence**
  – **Pathogenicity**
    • The ability of a microbe to cause disease
  – **Virulence**
    • The degree of pathogenicity in a microorganism

Virulence

• **Virulence** is determined by **invasiveness**, **toxicity**, and other factors produced by a pathogen.

• Various pathogens produce proteins that damage the host cytoplasmic membrane, causing cell lysis and death.

Measuring virulence

• **lethal dose 50 (LD₅₀)**
  – number of pathogens that will kill 50% of an experimental group of hosts

• **infectious dose 50 (ID₅₀)**
  – number of pathogens that will infect 50% of an experimental group of hosts
Pathogenesis of Bacterial Diseases

- initially evade host defenses
  - Some pathogenic bacteria are inherently able to resist the bactericidal components of host tissues.
  - Most successful pathogens, however, possess additional structural or biochemical features which allow them to resist the main lines of host defense
- damage host
  - The ability to invade tissues
  - The ability to produce toxins
- leave host and return to reservoir or enter new host

Toxigenicity

- intoxications
  - diseases that result from entry of a specific preformed toxin into host
- toxin
  - specific substance that damages host
- toxemia
  - condition caused by toxins in the blood of host
Exotoxins

• The most potent biological toxins are the **exotoxins** produced by microorganisms.

• Each exotoxin affects specific host cells, causing specific impairment of a major host cell function.

<table>
<thead>
<tr>
<th>Exotoxin</th>
<th>Producing Organism</th>
<th>Disease</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria toxin</td>
<td>Corynebacterium diptheriae</td>
<td>Diphtheria</td>
<td>Inhibits protein synthesis; affects heart, nerve tissue, liver</td>
</tr>
<tr>
<td>Botulism toxin</td>
<td>Clostridium botulinum</td>
<td>Botulism</td>
<td>Neurotoxin; fascicul paralysis</td>
</tr>
<tr>
<td>Perfringens</td>
<td>Clostridium perfringens</td>
<td>Gas gangrene</td>
<td>Hemolysin, collagenase, phospholipase</td>
</tr>
<tr>
<td>Erythrogenic toxin</td>
<td>Streptococcus pyogenes</td>
<td>Scarlet fever</td>
<td>Capillary destruction</td>
</tr>
<tr>
<td>Pyrogenic toxin</td>
<td>Staphylococcus aureus</td>
<td>Toxic shock syndrome</td>
<td>Fever, shock</td>
</tr>
<tr>
<td>Exfoliative toxin</td>
<td>Staphylococcus aureus</td>
<td>Scalded skin</td>
<td>Massive skin peeling</td>
</tr>
<tr>
<td>Exotoxin A</td>
<td>Pseudomonas aerugiosus</td>
<td>—</td>
<td>Inhibits protein synthesis</td>
</tr>
</tbody>
</table>

Roles of exotoxins in disease

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AB exotoxins

- composed of two subunits
  - A subunit – responsible for toxic effect and acts on the host cell
  - B subunit – binds to target cell
- Example: Tetanus toxin and botulinum toxin, both target neurons
- Example: Cholera toxin, target intestinal cells

Tetanus

- caused by Clostridium tetani
  - anaerobic, gram-positive spore-former
    - endospores found in soil, dust, hospital environments, and mammalian feces
    - produces tetanospasmin in low oxygen tension environments
    - causes prolonged muscle spasms
    - also produces tetanolysin, a hemolysin
- portal of entry – skin wounds

Tetanus

- clinical manifestations
  - early in disease – tension or cramping and twisting of skeletal muscles and tightness of jaw muscle
  - advanced disease – trismus (“lockjaw”), characteristic facial expressions, board-like rigidity of trunk, tonic convulsions, and backward bowing of back
  - death usually results from spasms of diaphragm and intercostal respiratory muscles
the action of tetanus toxin

from *Clostridium tetani*.

Tetanus

- treatment, prevention, and control
  - clinical history of wound infection and muscle stiffness
  - antibiotic therapy and treatment with antitoxin
  - active immunization with toxoid (DPT vaccine), and proper care of wounds contaminated with soil

Botulinum toxin

- Botulinum toxin consists of seven related toxins that are the most potent biological toxins known.

Membrane-disrupting exotoxins

Destroy integrity of eukaryotic membranes Cytolytic toxin

pore-forming exotoxins

- Phospholipases

phospholipases
Some pore-forming exotoxins

- Hemolysins: kill erythrocytes (red blood cells), leukocytes, and many other cells

Endotoxins

- **Endotoxins** are lipopolysaccharides derived from the outer membrane of gram-negative *Bacteria*. Released upon lysis of the *Bacteria*, endotoxins cause fever and other systemic toxic effects in the host.

- Endotoxins are generally less toxic than exotoxins

### Microbial Mechanisms for Escaping Host Defenses

#### Table 25.5: Properties of exotoxins and endotoxins

<table>
<thead>
<tr>
<th>Property</th>
<th>Exotoxin</th>
<th>Endotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical properties</td>
<td>Presence, exerted by most gram-positive or gram-negative bacteria, generally host-tissue specific.</td>
<td>Excretion-borne lipopolysaccharides (LPS) triggers LPS and LPS-dependent responses.</td>
</tr>
<tr>
<td>Mode of action; symptoms</td>
<td>Specific, usually local to specific cell receptors, or structures, other cytokines, endotoxins, or neutrophils</td>
<td>Endotoxins are highly toxic, systemically fatal.</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Usually highly toxic, sometimes fatal.</td>
<td>(LPS) triggers the production of neutralizing antibodies, stimulates immune responses.</td>
</tr>
<tr>
<td>Immunogenicity response</td>
<td>Highly immunogenic, stimulating the production of neutralizing antibodies (antitoxin).</td>
<td>( LPS ) is highly immunogenic, stimulates immune responses, but does not induce neutrophils or antibodies.</td>
</tr>
<tr>
<td>Toxic potential</td>
<td>Treatment of treated disease with formaldehyde, or trichloroethylene.</td>
<td>Nontoxic to host, but treated toxins (toxoids) induce immune responses.</td>
</tr>
<tr>
<td>Fever potential</td>
<td>Does not produce fever in host.</td>
<td>Pyrogenic; often induces fever in host.</td>
</tr>
</tbody>
</table>

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#### Table 26.5: Antiphagocytic factors produced by bacteria and their mode of action

<table>
<thead>
<tr>
<th>Factor</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocidins</td>
<td>Specific lytic agent for leukocytes including phagocytes.</td>
</tr>
<tr>
<td>Hemolysins</td>
<td>Form pores in host cells including macrophages. Streptolysin O affects sterols in membranes. Streptolysin S is a phospholipase.</td>
</tr>
<tr>
<td>Capsules (glycocalyx)</td>
<td>Long polymers of carbohydrate—physically prevents engulfment.</td>
</tr>
<tr>
<td>Fimbriae</td>
<td>(1) Bind to surface components of phagocytes, prevent close contact, and phagocytosis may not occur. (2) Phase variation—a change in the antigenic composition.</td>
</tr>
</tbody>
</table>
Host defenses

Normal flora: The human body is extensively colonized by bacteria (the normal flora). Some are mutualistic symbionts (e.g. intestinal bacteria synthesize vitamins).

Non-specific defenses:
- Physical barriers (e.g. skin: no known bacteria can penetrate the skin unaided!)
- Nutritional barriers: (e.g. lactoferrin/transferin tightly bind iron)

Specific defenses: Immune system

Physical, chemical, and anatomical barriers to infection

Normal Microbiota of the Human Body

- normal microbiota
  - microbes regularly found at an anatomical site

The Normal Flora of Humans

- Normal flora is present in
  - skin
  - upper respiratory tract
  - oral cavity
  - intestine, especially large intestine
  - vaginal tract
- Very little normal flora in eyes & stomach

The Normal Flora of Humans

- Notably absent in most all internal organs
  - Absent in:
    - lower respiratory tract
    - muscle tissue
    - blood & tissue fluid
    - cerebrospinal fluid
    - pericardium
The Normal Flora of Humans

- Benefits of the normal flora
  - Nutrient production/processing
    eg Vitamin K production by \textit{E. coli}
  - Competition with pathogenic microbes
  - Normal development of the immune system

Subject to Changes

- The number of different species of microorganisms; dynamic balance of many forces altering the bacterial population quantitatively as well as qualitatively
- Microbial composition; constantly changing
  - External influences
  - A direct result of the activities of the human host
- Influence by the other members of the ecosystem
- Intestinal microbial populations; diet, acidity of the stomach, ingestion of antibiotics, and peristalsis

Skin

- has both resident microbiota and transient microbiota
- mechanically strong barrier
- most areas subject to periodic drying
- slightly acidic
- salty
- inhibitory substances (e.g., lysozyme, cathelicidins)
- Primarily-gram positive bacteria—better adapted to dry condition than gram-negatives

The Human Gastrointestinal Tract

- Upper GI tract-
  Low pH,
  Sparse colonization
- Filamentous Microbes in the Ileum
- Large Intestine-
  A specialized fermentation vessel
Phagocytosis

Summary

Human-microbe interactions, including host-parasite (pathogen) are best understood from the point of view of microbial ecology. The human body is yet another habitat for microorganisms to colonize and with resources to exploit.

Pathogenesis occurs when a pathogen colonizes, invades, grows, and causes damage to the host tissue. Initial entry inoculum is usually small. Adherence factors are involved in invasion of specific tissues. Growth requires appropriate nutrients and environmental conditions. Nutrient availability is very important for pathogen success. Temperature, pH, oxygen concentration, etc. are important.

Pathogens produce a variety of virulence factors, such as enzymes/toxins that enhance virulence by breaking down or altering host tissue to provide access and nutrients. Still other pathogen-produced virulence factors provide protection to the pathogen by interfering with normal host defense mechanisms. These factors enhance colonization and growth of the pathogen.

Each region of the body provides different environmental conditions, and each selects for growth of a normal microbial flora.

Study Questions-21

1. How would one measure virulence?
2. Adherence is important in infection. Why?
3. What key features are shared by the A-B enterotoxins?
4. The B portions of A-B toxins are being used as vaccine components without any chemical treatment. Why is it safe to use B portions as they are?
6. How do the bacterial populations of the stomach and large intestine differ qualitatively and quantitatively? What role do the facultative aerobes play in the human gastrointestinal tract?