Lec. Microbiology **Date:6/11/2024**

Pathogenesis, virulence factors of bacterial pathogens

If a microorganism is capable of causing disease, it is called a **pathogen**. Fortunately, only a minority of the vast multitude of microorganisms in nature are pathogenic. Whereas some organisms are highly virulent and cause disease in healthy individuals, even with a small inoculum, others cause disease only in compromised individuals when their defenses are weak. The latter are called **opportunistic** organisms, as they take the opportunity offered by reduced host defenses to cause disease. These opportunists are frequently members of the body's normal flora.

General aspects of infection

Virulence

Virulence is a quantitative measure of pathogenicity and is related to an organism's **toxigenic potential** and **invasiveness**. Virulence can be measured by the number of organisms required to cause disease and is designated as LD50 or ID50: the LD50 (50% lethal dose) is the number of organisms needed to kill half the hosts, and ID50 (50% infectious dose) is the number needed to cause infection in half the hosts. These values are determined by inoculation of laboratory animals.

Communicable diseases

Infections are called 'communicable diseases' if they are spread from host to host. Many, but not all, infections are communicable; for example, tuberculosis is communicable, as it is spread by airborne droplets produced by coughing, but staphylococcal food poisoning is not, as the exotoxin produced by the organism and present in the contaminated food affects only those eating that food. If a disease is highly communicable, it is called a 'contagious disease' (e.g. chickenpox). Depending on the degree of incidence and prevalence of an infectious disease in a community, it may be called an endemic, an epidemic or a pandemic infection:

• An **endemic** infection is constantly present at a low level in a specific population (e.g. endemic malaria in some African countries).

• An infection is an **epidemic** if it occurs much more frequently than usual (e.g. an epidemic of influenza in the winter).

• An infection is a **pandemic** if it has a worldwide distribution (e.g. human immunodeficiency virus (HIV) infection and COVID- 19 infection).

Natural history of infectious disease

An acute infection generally progresses through four stages:

1. The **incubation period**: time between the acquisition of the organism or the toxin and the commencement of symptoms (this may vary from hours to days to weeks).

2. The **prodromal period**: non-specific symptoms such as fever, malaise and loss of appetite appear during this period.

3. The **acute specific illness**: the characteristic signs and symptoms of the disease are evident during this period.

4. The **recovery period**: the illness subsides and the patient returns to health during this final phase.

A number of organisms may elicit an **inapparent** or **subclinical** infection, without overt symptoms, where the individual remains asymptomatic although infected with the organism. On the other hand, once infected, the body may not completely eliminate the pathogen after recovery and some individuals may become **chronic carriers** of the organism (e.g. ***Salmonella typhi***, hepatitis B virus); they may shed the organism

while remaining healthy. Some infections result in a latent state, after which reactivation of the growth of the organism and recurrence of symptoms may occur at a later stage (e.g. after primary herpes infection, the virus may reside in a latent state in the trigeminal ganglion, causing recurrent herpes labialis from time to time). All the above groups may unknowingly shed pathogenic organisms and spread disease.

Pathogenesis of bacterial disease

Determinants of bacterial pathogenicity

Bacterial pathogenicity is a vast subject. The following is a brief outline of the ways and means by which bacteria cause disease. The major steps are transmission, adherence to host surfaces, invasiveness and toxigenicity.

Transmission

Most infections are acquired by transmission from external sources; i.e. they are exogenous in origin. Others are caused by members of the normal flora behaving as opportunist pathogens; i.e. they are endogenous in origin. Transmission can be by:

- inhalation – the airborne route (example: TB or measles)
- ingestion – fecal contamination of food and water ex: Cholera and Hepatitis A
- inoculation – contaminated needles
- sexual contact ex: Syphilis and Gonorrhea
- skin contact,
- blood transfusions
- biting insects such as malaria
- perinatal transmission ex: HIV, CMV….

There are four important portals of entry of pathogens:

1. skin
2. respiratory tract
3. gastrointestinal tract
4. genitourinary tract.

Adherence to host surfaces

Adherence is the first step in infection. Unless organisms have the ability to stick or adhere to host surfaces, they will be unable to cause infection. Some bacteria and fungi have specialized structures or produce substances that facilitate their attachment to the surface of human cells or prostheses (e.g. dentures, artificial heart valves), thereby enhancing their ability to colonize and cause disease. These adherence a mechanisms are critical for organisms that attach to mucous membranes; mutants that lack these mechanisms are often non-pathogenic (e.g. the hair-like pili of *Neisseria gonorrhoeae* and *Escherichia coli* mediate their attachment to the urinary tract epithelium; the extracellular polysaccharides of *Streptococcus mutans* help it adhere to enamel surfaces).

Biofilm formation

Once the organisms adhere to a host surface they usually tend to aggregate and form intelligent communities of cells called **biofilms**. A biofilm is defined as an aggregate of interactive bacteria attached to a solid surface (such as a denture prosthesis or an intravenous catheter) or to each other, encased in an extracellular polysaccharide matrix. Up to 65% of human infections are thought to be associated with microbial biofilms. **Dental plaque** on solid enamel surfaces is a classic example of a biofilm. As biofilms are ubiquitous in nature and form on hulls of ships, warm water pipes, dental unit water systems and so on.

As mentioned, biofilms are intelligent communities. Structurally, they are not flat and compressed but comprise a complex architecture with towers and mushroom or dome shaped structures with water channels that permit transport of metabolites and nutrients. Bacteria in biofilms maintain the population level by constantly secreting low levels of chemicals called **quorum-sensing molecules** (e.g. homoserine lactone), which tend to repulse incoming bacteria or activate the communal bacteria to seek new abodes. Further, specific gene activation may lead to production of virulence factors or reduction in metabolic activity (especially those living deep within the matrix). It is now known that infections associated with biofilms are difficult to eradicate as **sessile organisms** in biofilms exhibit higher resistance to antimicrobials than their free living or **planktonic** counterparts. The reasons for this appear to be:

• protection offered by the extracellular polysaccharide matrix from the host immune mechanisms
• poor penetration of the antimicrobials into the deeper layers of the biofilm
• degradation of the antimicrobials as they penetrate the biofilm
• difference in pH and redox potential (*E*h) gradients that is not conducive for the optimal activity of the drug
• gene expression leading to more virulent or resistant organisms.

Some examples of important recalcitrant human infections mediated by biofilms, difficult to manage by antimicrobials alone, include *Pseudomonas aeruginosa* infections of the respiratory tract in cystic fibrosis patients, *Staphylococcus aureus* in central venous catheters, chronic candida infections of HIV-infected individuals and chronic periodontal infections due to dental plaque.

Invasiveness (virulence factors)

Invasiveness of bacteria plays a critical role in pathogenesis; this property is dependent upon secreted bacterial enzymes. A few examples are:

• **Collagenase** and **hyaluronidase** degrade their respective intercellular substances, allowing easy spread of bacteria through tissues, and are especially important in skin infections caused by *Streptococcus pyogenes*.

• **Coagulase**, produced by *Staphylococcus aureus*, accelerates the formation of a fibrin clot (from fibrinogen). It helps protect the organisms from phagocytosis by walling off the infected area and by coating the organisms with a fibrin layer.

• **Immunoglobulin A (IgA) protease** degrades protective IgA on mucosal surfaces, allowing organisms such as *N. gonorrhoeae, Haemophilus influenzae* and *Streptococcus pneumoniae* to adhere to mucous membranes.

• **Leukocidins** can destroy both neutrophilic leukocytes and macrophages; the periodontopathic organism *Aggregatibacter actinomycetemcomitans* possesses this enzyme. The mutants that do not secrete the enzyme are less virulent.

Other factors also contribute to invasiveness by interfering with the host defence mechanisms, especially phagocytosis:

• The polysaccharide **capsule** of several common pathogens, such as *Streptococcus pneumoniae* and *Neisseria meningitidis*, prevents the phagocyte from adhering to the bacteria. (This can be verified by the introduction of anticapsular antibodies, which allow more effective phagocytosis or opsonization to occur. Thus, the vaccines against *Streptococcus pneumoniae* and *N. meningitidis* contain capsular polysaccharides that induce protective **anticapsular antibodies**.)

• The **cell wall proteins** of the Gram-positive cocci, such as the M protein of the group A streptococci and protein A of the staphylococci, are also antiphagocytic.

Bacterial infection may lead to two categories of inflammation: pyogenic (pus-producing) and granulomatous (granuloma-forming).

Pyogenic inflammation

The neutrophils are the predominant cells in this type of inflammation. *Streptococcus pyogenes*, *Staphylococcus aureus* and *Streptococcus pneumoniae* are the common pyogenic bacteria.

Granulomatous inflammation

Macrophages and T cells predominate in this type of inflammation. The most notable organism in this category is *Mycobacterium tuberculosis*. Here, the bacterial antigens stimulate the cell-mediated immune system, resulting in sensitized T-lymphocyte and macrophage activity. Although the phagocytic activity of macrophages kills most of the tubercle bacilli, some survive and grow within these cells, leading to **granuloma formation**. The organisms reside within **phagosomes**, which are unable to fuse with lysosomes, resulting in protection from degradative enzymes therein. Many fungal diseases are also characterized by granulomatous lesions.

Toxigenicity

Toxin production or toxigenicity is another major mediator of bacterial disease. Toxins are of two categories: **endotoxins** and **exotoxins**.

Toxin production

Endotoxins

Endotoxins are the cell wall lipopolysaccharides of Gram negative bacteria and are not actively released from the cell. (Note: thus, by definition, Gram positive organisms do not possess endotoxins.) Endotoxins cause fever, shock and other generalized symptoms. A number of biological effects of endotoxin are described below. These are mainly due to the production of host factors such as **interleukin-1 (IL-1**) and **tumour necrosis factor (TNF)** from macrophages.

1. Fever is due to the release of endogenous pyrogens (IL-1) by macrophages; these act on the hypothalamic temperature regulatory centre and reset the 'thermostat' at a higher temperature.

2. Hypotension, shock and reduced perfusion of major organs due to vasodilatation, are brought about by bradykinin release, increased vascular permeability and decreased peripheral resistance.

3. Activation of the **alternative pathway of the complement cascade** results in inflammation and tissue damage.

4. Generalized **activation of the coagulation system** (via factor XII) leads to disseminated intravascular coagulation (DIC), thrombosis and tissue ischaemia.

5. There is **increased phagocytic activity** of macrophages and polyclonal B cell activation (but not T lymphocytes).

6. There is **increased antibody production**.

Endotoxin-like effects may also occur in Gram-positive bacteraemic infections. However, as endotoxin is absent in Gram-positive bacteria, other cell wall components, such as teichoic acid or peptidoglycan, are thought to trigger the release of TNF and IL-1 from macrophages.

Exotoxins

Both Gram-positive and Gram-negative bacteria secrete exotoxins, whereas endotoxin is an integral component of the cell wall of Gram-negative organisms. Exotoxins in particular can cause disease in distant parts of the body as a result of diffusion or carriage of the toxin via systemic routes (e.g. tetanus bacillus infecting a lesion in the foot produces an exotoxin, which causes 'lockjaw' or spasm of masseter muscles on the face).

Exotoxins are polypeptides, these polypeptides consist of two domains or subunits: one for binding to the cell membrane and entry into the cell, and the other possessing the toxic activity.
Exotoxins are highly toxic (e.g. the fatal dose of tetanus toxin for a human can be less than 1 μg). Fortunately, exotoxin polypeptides are good antigens and induce the synthesis of protective antibodies called **antitoxins**, useful in the prevention or treatment of diseases such as tetanus. The toxicity of the polypeptides can be neutralized when treated with formaldehyde (or acid or heat), and these **toxoids** are used in protective vaccines because they retain their antigenicity.
Bacterial exotoxins can be broadly categorized as:

- neurotoxins
- enterotoxins
- miscellaneous exotoxins.

Neurotoxins

Tetanus toxin, diphtheria toxin and botulinum toxin are all neurotoxins and their action is mediated via neuronal pathways. **Tetanus toxin**, produced by *Clostridium tetani*, is a neurotoxin that prevents the release of the inhibitory neurotransmitter glycine, thus causing muscle spasms, Tetanus toxin (tetanospasmin) comprises two polypeptide subunits: a heavy chain and a light chain. The former binds to the gangliosides in the membrane of the neuron, while the latter is the toxic component. The toxin is liberated at the peripheral wound site but is transmitted to the neurons of the spinal cord either by retrograde axonal transport or in the blood stream. There it blocks the release of the inhibitory transmitter, which leads to sustained and convulsive contractions of the voluntary muscles (e.g. *risus sardonicus*, contraction of the facial muscles; lockjaw, contraction of the masseter muscles).

Diphtheria toxin, produced by *Corynebacterium diphtheriae*, is synthesized as a single polypeptide with two functional domains. Once secreted, one domain mediates the binding of the toxin to cell membrane receptors; the other domain possesses enzymatic activity and inhibits protein synthesis in all eukaryotic cells. The enzyme activity is highly potent: a single molecule can kill a cell within a few hours. *E. coli*, *Vibrio cholerae* and *Bordetella pertussis* also possess exotoxins that act in a similar manner.
Botulinum toxin, produced by *Clostridium botulinum*, is one of the most toxic compounds known (1 μg will kill a human). The toxin blocks the release of acetylcholine at the synapse, producing paralysis of both voluntary and involuntary muscles.

Enterotoxins

These toxins act on the gut mucosa and cause gastrointestinal disturbances. *E. coli* enterotoxin is of two types: one heat labile and one heat stable. The heat-labile toxin (inactivated at 65°C in 30 min) is composed of two domains: one binds to a ganglioside in the cell membrane, while the other is the active component and mediates synthesis of cyclic adenosine monophosphate (cAMP) in the mucosal cells of the small intestine. This leads to an increase in the concentration of cAMP, which promotes cellular chloride ion excretion and inhibition of sodium ion absorption. The net result is fluid and electrolyte loss into the lumen of the gut (diarrhoea). The heat-stable toxin of *E. coli* (not inactivated by boiling for 30 min) stimulates guanylate cyclase and thus increases the concentration of cyclic guanosine monophosphate (cGMP), which inhibits the reabsorption of sodium ions and causes diarrhoea (compare with heat-labile toxin). The genes for both toxins are carried on a plasmid. The enterotoxins produced by the diarrhoea-causing organisms *V. cholerae* and *Bacillus cereus* act in a manner similar to that of the heat-labile toxin of *E. coli*.

Reference:

BOOK: Samaranayake L. (2012). Essential microbiology for dentistry.Pp:37-41. Elsevier Ltd. Fourth edition.

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