Lecture (2)

Classification of Exotoxins: -

The term ‘host cell’ refers to either vertebrate cells or cells of lower eukaryotes, such as protozoa, because some bacterial exotoxins intoxicate a broad range of host cells. The recognition that some pathogenic bacteria produced soluble components capable of producing the pathology associated with a particular disease was determined in the late nineteenth century. Roux and Yersin observed that culture filtrates of *Corynebacterium diphtheriae* were lethal in animal models and that the pathology elicited by the culture filtrate was similar to that observed during the infection by the bacterium. Subsequent studies isolated a protein, diphtheria toxin, from the toxic culture filtrates and showed that the administration of purified diphtheria toxin into animals was sufficient to elicit the pathology ascribed to diphtheria. Diphtheria toxin is a prototype exotoxin and has been used to identify many of the biochemical and molecular properties of bacterial exotoxins.

The ability of a bacterial pathogen to cause disease frequently requires the production of exotoxins, but the mere ability to produce a toxin is not sufficient to cause disease. Cholera toxin is the principal virulence factor of *Vibrio cholerae*. Administration of micrograms of purified cholera toxin to human volunteers elicits a diarrheal disease that mimics the magnitude of the natural infection. Nonetheless, nonvirulent toxin-producing strains of *V. cholerae* have been isolated and shown to lack specific biological properties, such as motility or chemotaxis. Similarly, although anthrax toxin is the principal toxic component of

Bacillus anthracis, nonvirulent toxin-producing strains of *B. anthracis* have been isolated and shown to lack the ability to produce a polyglutamic acid capsule. An exception to this generalization is the intoxication elicited by the botulinum neurotoxins, in which ingestion of the preformed toxin is responsible for the elicitation of disease; food poisoning by botulinum neurotoxins is an intoxication rather than an infection by a toxin-producing strain of *Clostridium botulinum*.

Bacterial exotoxins are classified according to their mechanisms of action. The covalent modifications of host cell components, which are catalyzed by bacterial exotoxins, include ADP-ribosylation, deamidation, depurination, endoproteolysis, and glucosylation. Most cellular targets of bacterial exotoxins are proteins, although there are exceptions such as Shiga toxin, which catalyzes the deadenylation of ribosomal RNA. In addition to exotoxins, there are several other classes of toxins that are produced by bacterial pathogens, including the poreforming toxins, type III-secreted cytotoxins, heat-stable enterotoxins, and superantigens. Each of these toxins fails to perform one of the properties associated with exotoxins. The pore-forming toxins are not catalytic in their action but instead disrupt cell physiology through the formation of pores in the host cell plasma membrane. The type III secreted cytotoxins cannot enter host cells as soluble proteins but instead are injected directly into the host cell by the type III secretion apparatus of the cell-bound bacterium. The heat-stable enterotoxin and superantigens do not enter the intracellular compartment of the host cell and elicit host cell responses by triggering signal transduction pathways upon binding to the host cell membrane.

Exotoxins are soluble, heat-labile, proteins (a few are enzymes) that usually are released into the surroundings as the bacterial pathogen grows. Often exotoxins may travel from the site of infection to other body tissues or target cells in which they exert their effects. Exotoxins usually are: -

1. Synthesized by specific bacteria that often have plasmids or prophages bearing the exotoxin genes.
2. Heat-labile proteins inactivated at 60 to 80°C.
3. Among the most lethal substances known (toxic in very small doses [microgram per kilogram amounts]; e.g., the botulinum toxin).
4. Associated with specific diseases and have specific mechanisms of action.
5. Highly immunogenic and stimulate the production of neutralizing antibodies called antitoxins.
6. Easily inactivated by formaldehyde, iodine, and other chemicals to form immunogenic toxoids.
7. Unable to produce a fever in the host directly.
8. Often given the name of the disease they produce (e.g., the diphtheria toxin).

Exotoxins can be divided into four types based on their structure and physiological activities.

(1) One type is the AB toxin, which gets its name from the fact that the portion of the toxin (B) that binds to a host cell receptor is separate from the portion (A) that has the enzyme activity that causes the toxicity.

(2) A second type, which also may be an AB toxin, consists of those toxins that affect a specific host site (nervous tissue [neurotoxins], the intestines [enterotoxins], general tissues [cytotoxins]) by acting extracellularly or intracellularly on the host cells.

(3) A third type does not have separable A and B portions and acts by disorganizing host cell membranes. Examples include the leukocidins, hemolysins, and phospholipases.

(4) A fourth type is the superantigen that acts by stimulating T cells to release cytokines.

AB Exotoxins. AB toxins are composed of an enzymatic subunit or fragment (A) that is responsible for the toxic effect once inside the host cell and a binding subunit or fragment (B). Isolated A subunits are enzymatically active but lack binding and cell entry capability, whereas isolated B subunits bind to target cells but are nontoxic and biologically inactive. The B subunit interacts with specific receptors on the target cell or tissue such as the gangliosides GM1 for cholera toxin, GT1 and/or GD1 for tetanus toxin, and GD1 for botulinum toxin.

Several mechanisms for the entry of A subunits or fragments into target cells have been proposed. In one mechanism the B subunit inserts into the plasma membrane and creates a pore through which the A subunit enters. In another mechanism entry is by receptor-mediated endocytosis. The mechanism of action of an AB toxin can be quite complex, as shown by the example of diphtheria toxin. The diphtheria toxin is a protein of about 62,000 mol wt. It binds to cell surface receptors by the B fragment portion and is taken into the cell through the formation of a clathrin-coated vesicle. The toxin then enters the vesicle membrane and is cleaved into two parts, one of which, the A fragment, escapes into the cytosol. The A fragment is an enzyme that catalyzes the addition of an ADP-ribose group to the eucaryotic elongation factor EF2

that aids in translocation during protein synthesis. The substrate for this reaction is the coenzyme NAD+.

The modified EF2 protein cannot participate in the elongation cycle of protein synthesis, and the cell dies because it can no longer synthesize proteins.

AB exotoxins vary widely in their relative contribution to the disease process with which they are associated.

Genetic Organization of Exotoxins: -

The genes encoding bacterial exotoxins may be located on the chromosome or located on an extrachromosomal element, such as a plasmid or a bacteriophage. Elegant experiments characterizing diphtheria toxin showed that the gene encoding this exotoxin was located within the genome of the lysogenic phage. Although both nonlysogenic and lysogenic strains of *C. diphtheriae* could establish local upper respiratory tract infection, only strains of *C. diphtheriae* lysogenized with _-phage that encoded diphtheria toxin were capable of eliciting systemic disease.

Most exotoxins are produced only during specific stages of growth, with the molecular basis for the regulation of toxin expression varying with each bacterium. This differential expression often reflects a complex regulation of transcription, including responses to environmental conditions, such as iron. Multisubunit toxins are often organized in operons to allow the coordinate expression of their subunit components.